JOURNAL OF DAIRY SCIENCE

Contents

A Breed Comparison in the Vitamin D Content of Milk with Notes on a Modified Technique for the Vitamin D Assay of Low-Potency Fats and Oils. G. C. WALLIS 733

Lactic Acid in Dairy Products. I. Application of the Hillig Method. I. A. GOULD 743

Lactic Acid in Dairy Products. II. Relation to Flavor, Acidity Measurements, Bacterial Count and Methylene Blue Reduction. I. A. GOULD and J. M. JENSEN 753

A Technique for the Simultaneous Measurement of Semen Quality and Testis Histology in Vitamin A Studies of the Dairy Bull. R. E. ERB, F. N. ANDREWS, J. F. BULLARD and J. H. HILTON 769

Seasonal and Geographical Variations in the Citric Acid Content of Defatted Milk Solids. BURDET HEINEMANN 773

Further Studies on Bloat. S. W. MEAD, H. H. COLE and W. M. REGAN 779

Non-Permeability of the Mammary Gland to Thyroid Hormone. E. P. REINEKE and C. W. TURNER 793

Abstracts of Literature A119

Vol. XXVII, No. 9, September, 1944

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION

THE Duraglas HANDI-QUART

TRADE MARK REG. U. S. PAT. OFF.

Saves on Milk Delivery Because It's a Better *Glass* Container

The *GLASS* container is the standard for economical milk delivery. It is returnable. It can be sterilized easily and safely. It is the trustworthy container that delivers milk in all kinds of weather. It is the faithful milk container customers prefer.

The only way to improve this tested milk delivery system is to improve the *GLASS* milk bottle. Owens-Illinois has done this with the DURAGLAS Handi-Quart, a tough, rugged milk bottle that gives high trippage. And remember that only Duraglas milk bottles have the *Syncro-Flash** finish for greater resistance to impact, better capping, easier cleaning.

**Reg. U. S. Pat. Off.*

OWENS-ILLINOIS *Complete Dairy Service*

TOLEDO OHIO

WHERE QUALITY PREDOMINATES

Your advertisement is being read in every State and in 25 Foreign Countries

STERI-CHLOR

a little kills a lot of germs

DISHING out death to bacteria—on equipment, utensils, containers, and wherever they're found in your plant—is an important job you can safely entrust to Wyandotte Steri-Chlor,* the easy-to-use but powerful germicide.

Check off these advantages of Wyandotte Steri-Chlor, and see if they don't add up to the perfect solution to sanitation—all along the line.

- Effective at both high and low temperatures.
- Has no bleaching action.
- Safe on metals.
- It leaves no odor or taste after use.
- Helps prevent formation of milkstone.
- Its low alkalinity and almost neutral pH provide more effective germicidal action.
- Does not lose strength when left in opened container.
- Makes a clear solution in hard or soft water.
- The chlorine is *all* available, without acidifying, when dissolved in water.

*Registered trade-mark

Make it a point to ask your Wyandotte Representative about specific applications of Wyandotte Steri-Chlor in your plant. Let him help you put this powerful germicide to work.

WYANDOTTE CHEMICALS CORPORATION
J. B. FORD DIVISION • WYANDOTTE, MICHIGAN
SERVICE REPRESENTATIVES IN 88 CITIES

Wyandotte
REG. U. S. PAT. OFF

Your advertisement is being read in every State and in 25 Foreign Countries

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION
Published at
NORTH QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.

T. S. SUTTON, Editor
Columbus, Ohio

Associate Editors

O. F. HUNZIKER Chicago, Ill.	C. A. CARY Beltsville, Md.	PAUL F. SHARP Piedmont, Calif.
C. E. GRAY San Francisco, Cal.	E. G. HASTINGS Madison, Wis.	H. A. RUEHE Chicago, Ill.
I. A. GOULD East Lansing, Mich.	I. W. RUPEL Madison, Wis.	P. R. ELLIKER Lafayette, Ind.

Committee on Journal Management
FORDYCE ELY, *Chairman*

G. E. HOLM
T. S. SUTTON, *ex officio*
G. M. TROUT
R. B. STOLTZ, *ex officio*

Subscriptions. Price; $6.00 per volume in North and South America; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Subscriptions for the British Isles and British Empire, except for Canada and Australia, should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert St., St. Leonards, N. S. W., Australia.

Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

OFFICERS OF THE ASSOCIATION

A. C. RAGSDALE, President
Columbia, Missouri

J. A. NELSON, Vice-President
Bozeman, Montana

R. B. STOLTZ, Sec.-Treas.
Columbus, Ohio

DIRECTORS

P. H. TRACY Urbana, Illinois	W. E. PETERSEN St. Paul, Minn.	P. A. DOWNS Lincoln, Nebr.
C. L. BLACKMAN Columbus, Ohio	R. B. BECKER Gainesville, Fla.	J. W. LINN Manhattan, Kans.
	A. C. DAHLBERG Ithaca, New York	

Entered as second-class matter April 13, 1934 at the postoffice at Lancaster, Pa., under the act of March 3, 1879.

FIRST

FOR CONTROL IN DAIRY PRODUCTION AND QUALITY

ON AMERICA'S GREATEST FOOD FRONT...

For Assurance

DISTRIBUTED BY LEADING DAIRY SUPPLY DEALERS THROUGHOUT THE UNITED STATES AND CANADA

• • • *The Visible Guarantee of Invisible Quality* • • •

Your advertisement is being read in every State and in 25 Foreign Countries

DE LAVAL HAS THE WINNING MILKING COMBINATION FOR YOU

DE LAVAL MILKERS

DE LAVAL MAGNETIC SPEEDWAY MILKER

The De Laval Magnetic Speedway Milker

The De Laval Magnetic Speedway is different and better in principle and performance. It is the only milker having pulsations for each unit created and controlled at the pulso-pump by magnetic force and assuring perfect uniformity of milking. The cows are always milked in the same perfect way.

In every respect the De Laval Magnetic Speedway is the world's best milker.

The De Laval Sterling Milker

The De Laval Sterling provides De Laval quality milking for small herds. The wonderful Sterling Pulsator has only two moving parts and provides positive, precise milking speed and action that pleases the cow.

De Laval Sterling Units can also be used on any other make of single pipe line installation to obtain De Laval quality milking.

DE LAVAL STERLING MILKER

PLUS . . . THE DE LAVAL *SPEEDWAY* METHOD OF FAST MILKING

The De Laval Speedway Method of Fast Milking is a simple, common-sense milking routine which (1) saves time (2) promotes better udder health and (3) improves milk production.

It is now widely used, with more and more dairy farmers changing their herds over to it each day.

It has the approval of leading dairy authorities —many of the state agricultural colleges through their extension services and the county agents are teaching fast milking through meetings and demonstrations.

Fast Milking brings *improved* results with *any* method of milking—but *best* results with De Laval Milkers.

It's simple, easy and practical. Good results show up at once. Try it on your own herd.

THE DE LAVAL *SPEEDWAY* METHOD OF FAST MILKING

1. ***Be regular***—Start the milking at the same time each milking.

2. ***Have everything in readiness***—Avoid unnecessary noise, confusion or distraction of any kind in the barn at milking time. Study your milking routine to eliminate every unnecessary move.

3. ***Preparation of the cow***—Thoroughly wipe the udder of each cow, just before it is her turn to be milked, with a clean cloth which has been immersed in warm water (130° F.) containing 250 parts per million of available chlorine. Follow immediately with Step 4.

4. ***Use of the Strip Cup***—Next, using a full hand squeeze, draw a few streams of milk from each quarter into strip cup. Inspect for abnormal milk; if present, milk cow last. (Steps 3 and 4 induce rapid let-down of the milk.)

5. ***Apply teat-cups immediately after using Strip Cup***—Hold and apply teat-cups properly so that no vacuum is lost and least amount of air is admitted.

6. ***Teat-cups should be removed from cow at end of 3 to 4 minutes***—Hand stripping should be employed chiefly for purposes of inspection, and should consist of only a few full hand squeezes from each quarter. Do not prolong hand stripping. Machine stripping can be done just before removing teat-cups by massaging each quarter briefly.

THE DE LAVAL SEPARATOR COMPANY

New York 6	Chicago 6	San Francisco 19
165 Broadway	427 Randolph St.	61 Beale St.

Your advertisement is being read in every State and in 25 Foreign Countries

To their GOOD HEALTH!

IT'S going to take healthy soldiers, healthy war workers, healthy families and healthy communities to win this war. That's the big reason why today it's more important than ever before to maintain highest standards in the purity of the milk they drink, despite the growing problems of changing conditions and changing personnel.

And SEAL-KAPS help to maintain high standards. For SEAL-KAPS not only tightly cover the pouring lip of the bottle from dairy to delivery but they *re*-cover the bottle after every use. Thus the milk is constantly guarded against the danger of contamination and pure milk is kept pure to the very last drop.

AMERICAN SEAL-KAP CORPORATION
11-05 44th Drive, Long Island City, N. Y.

SEAL-KAP COVERS THE DANGER ZONE

The Bad Boys Are on the Spot

IN DEMOCRATIC countries, police departments nab "bad actors" and keep them out of circulation. In food processing, we also encounter "bad actors"—elements and conditions that rob products of freshness, turn them rancid, destroy their flavor. But food technologists are already on their trail. They know, for example, that they must protect food products from irradiation by light . . . exclude oxygen or excessive humidity . . . guard against insect infestation.

Recent research shows that copper and iron are "bad actors." In the manufacture of shortening, they are eliminated to increase rancidity resistance of fats and oils. They affect stability of practically any food that contains fats or volatile flavors easily oxidized.

Yes, they even affect the stability of Vitamin "C" and some of the "B" group.

But we have reduced the copper and iron in Diamond Crystal Salt until today they represent less than 1 part per million. Salt products in general, however, may contain significant amounts of both copper and iron.

NEED HELP? HERE IT IS!

Why not check up on the salt you are now using? And if you would like help in keeping down the "bad actors," copper and iron, in your plant, write to Technical Director, Diamond Crystal, Dept. H-3, St. Clair, Michigan.

DIAMOND CRYSTAL SALT
ALBERGER PROCESS

Your advertisement is being read in every State and in 25 Foreign Countries

More than 6000 Years of "KNOW HOW" in CHERRY-BURRELL FACTORIES

THREE hundred thirty-seven employees in Cherry-Burrell *factories* have a total of over 6000 years of "know how" in building dependable dairy plant equipment. Many of them have worked with us "boy and man" for 45 years or more. They and those who work with them are skilled craftsmen in designing and building Cherry-Burrell quality equipment. They "know how."

And That Isn't All

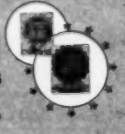
There are dozens of other veterans like these. You will find them among Cherry-Burrell *salesmen, branch managers, office and warehouse folks.* Many of these men and women, too, have spent their business lives serving the dairy industry. Notice the Service Emblems they proudly wear. They "know how" too.

Dozens more will join the ranks of these veterans this year, and the next and the next. Cherry-Burrell "know how" is accumulating for your benefit, year after year.

All this is mighty important to you who want the best in plant equipment, dependable advice and conscientious service. It means that when you are ready to plan the layout for a processing department, or an entirely new plant . . . or whenever you need dependable advice on equipment problems, it will pay you to call the Cherry-Burrell man. The "know how" of this great organization of veterans is ready to work with him and through him, for you.

Complete Equipment and Supplies for Handling Milk and its Products

CHERRY-BURRELL CORPORATION
427 WEST RANDOLPH STREET, CHICAGO 6, ILLINOIS
★ ★ ★
FACTORIES, WAREHOUSES, BRANCHES, OFFICES OR DISTRIBUTORS AT YOUR SERVICE IN 93 CITIES



. . . and Horace was right!

QUINTUS HORATIUS FLACCUS
65-8 B. C.

Nearly 2000 years ago Horace said:—"Sincerum est nisi vas quodcumque infundis acescit".

"Unless the vessel be clean—whatever you pour into it turns sour"

Right this very day too many tons of valuable and badly needed milk were wasted because somebody forgot what Horace knew back in 20 B. C.

Right this very day too much milk was wasted because somebody poured it into a dirty vessel and far too often that vessel was a milking machine that had not been scrubbed clean. Some of those dirty vessels were doubtless Surge Milkers.

After all the labor and care and time and expense that has gone into the production of a can of milk it does seem a shame to waste the whole thing for lack of an extra two minutes used in making sure that the milking machine is clean.

Keeping things clean by scrubbing them clean is the tiniest part of the cost of milk production. Scrubbing costs very little in either time or money.

The
SURGE MILKER

We have printed the following words literally millions of times. We try very hard to expose every Surge User to them several times a year.

> *"In order to produce clean milk, the machine must be washed clean after every milking—not simply rinsed and placed in a strong sterilizing solution. You cannot buy any magic compound that will take the place of thorough washing. It takes a clean machine to produce clean milk; don't let anybody tell you different."*

We have nothing whatever against the wetting agents or any of the other new and useful preparations that have been so widely recommended for the cleaning and sterilization of milking machines. We wish that they were more widely distributed and used in greater quantities but we still have not seen the "magic compound" that would take the place of a thorough scrubbing with a proper brush.

Under laboratory conditions—certainly! On the average farm —NO!

Let's keep remembering that the average farmer does not have an abundance of hot water and for a long time to come he is not going to have plenty of hot water. A magic compound that depends upon "plenty of hot water" can't quite solve our problems.

The Surge Milker has no long tubes and no milk claws and it should therefore be easily scrubbed clean.

It *is* easily scrubbed clean but it still needs to be taken apart and scrubbed *every time it is used.*

1.—When the count is high . . . check the inflation first of all. They used to be made of rubber and are now made of synthetic compounds with which our experience is still limited. They are more porous than metal and the teat cup should be snapped apart and the inflations tossed into clean cold water . . . then *scrubbed* with hot alkali solution. They can be kept clean and sterile and in good condition if kept immersed in lye water that is fresh and up to strength.

2.—The lid nipple is easily scrubbed clean and seldom causes trouble.

3.—The Surge Check Valve works. It is almost impossible to contaminate the milk by any drainage back from the pipe line or long vacuum hose. Naturally, the part that the milk touches should be clean.

4.—The big nipple on the pulsator has nothing to do with high count milk . . . neither does the long vacuum hose or the pipe line. They should be *clean* . . . if they are not clean they will slow up milking but they won't put bacteria into the milk. The air from between the inflation and teat cup shell does not pass through the pail.

5.—The Surge pail is easy to brush clean. It has a glasslike smooth finish . . . there are no corners to trap milk solids . . . and . . . you never saw a rusty Surge pail. Only indifferent care can make it a bacterial hazzard to quality milk. Scrubbing it thoroughly clean is common sense insurance.

Twenty years of experience on many thousands of farms in many countries and an extreme range of conditions have satisfied us that the surest and most economical way to keep a Surge Milker clean is to snap it all apart and rinse it in cold water the second that it comes off the last cow.

Then . . . *SCRUB* it with such hot water as is a available plus an alkali. Some compounds are doubtless better than others but the worst one will get the job done if mixed with enough elbow grease.

Doing it that way does not take much time, is not a hardship and is thoroughly practical . . . it is being done daily by thousands of men in many countries . . . and it does aid farmers to produce better milk . . . than with any other method.

The more hot water there is available the easier and bettter the job but thousands of farmers have practically no hot water at all.

You may not like it . . . you may be very much against it but you might be surprised at the low counts you can get with a Surge Milker scrubbed and sterilized with nothing but ice water and sodium hydroxide . . . plain old household lye when it is properly used.

We are doing everything that we can think of to do to keep every Surge Milker everywhere clean and sterile . . . we ask your help.

Surge
DAIRY FARM EQUIPMENT

BABSON BROS. CO.

2843 West 19th St., Chicago 23, Ill.

Syracuse • Minneapolis • Kansas City • Los Angeles • Houston • Seattle

JOURNAL OF DAIRY SCIENCE

VOLUME XXVII SEPTEMBER, 1944 NUMBER 9

A BREED COMPARISON IN THE VITAMIN D CONTENT OF MILK WITH NOTES ON A MODIFIED TECHNIQUE FOR THE VITAMIN D ASSAY OF LOW-POTENCY FATS AND OILS*

G. C. WALLIS

Department of Dairy Husbandry, South Dakota Agricultural Experiment Station, Brookings, South Dakota

Information on breed differences in the vitamin D content of milk is decidedly meager. Bechtel and Hoppert (1) have reported an extensive study of seasonal variations of vitamin D in normal cow's milk. Holsteins and Guernseys were included in this study so some comparisons are possible between these two breeds. Normal herd management was employed so no information is available as to the actual vitamin D intake. Under these conditions the authors noted that there was little difference in the antirachitic potency of the milk fat of the two breeds. They pointed out that the higher percentage of fat in the Guernsey milk largely accounted for the higher vitamin D potency per quart of milk for this breed. The vitamin D values for the Guernsey milk varied from 4.8 to 43.8 U.S.P. units per quart whereas the extreme values for the Holstein milk were 3.1 to 27.7 U.S.P. units per quart. Kon and Henry (5) have reported a comparison between Guernsey and Shorthorn cows under pasture conditions with supplemental feeds as required. Under these conditions the actual intake could not be measured. The Guernsey butterfat contained 0.35 International Units per gram and the Shorthorn 0.28. The difference was not statistically significant so these authors also suggested that the vitamin D activity of the milk of the two breeds is in proportion to the fat percentage of the milk.

On the other hand, the recommendations for feeding irradiated yeast to dairy cows for the production of vitamin D milk with a uniform potency per quart called for a given amount of yeast for a given level of milk production (7). These recommendations were based on extensive research which seemed to indicate that differences in breed, fat percentage of the milk, and such factors were relatively insignificant. It is quite evident that if milk of varying butterfat content is to have the same vitamin D potency

Received for publication November 30, 1943.

* Approved for publication by the Director of the South Dakota Agricultural Experiment Station as Journal Series No. 181.

Copyrighted, 1944, by the AMERICAN DAIRY SCIENCE ASSOCIATION.

per quart there must be a compensating difference in the potency of each gram of fat in the milk. This relationship differs somewhat from the results of the investigations (1) and (5) already reviewed. Of course, these differences may result in part from the fact that normal herd management was used in one case while comparatively large supplements of vitamin D were used in the other. The experiments being reported in this paper were designed to gain more information on this problem and to study breed differences in the vitamin D content of milk using normal rations to supply constant, known amounts of vitamin D.

EXPERIMENTAL METHODS

Plan of main experiment. Holsteins and Jerseys were used in this experiment, thus providing a wide difference in the quantity and butterfat percentage of the milk produced. Animals, representative of the breeds concerned were carefully paired to eliminate as many variables as possible. Particular attention was given to have freshening dates close together for each pair so that seasonal and management differences would not affect the vitamin D values.

Three pairs of cows have been studied for one complete lactation. An attempt was made to equalize the vitamin D reserves of each pair by either placing them on a vitamin-D-deficient ration for a preliminary period of two months or by feeding the experimental ration with a known vitamin D content for two or three months before freshening. Alfalfa hay furnished the only source of vitamin D. The cows were exercised after dark or in a shed. Sufficient hay was secured at the beginning to feed each pair throughout the entire lactation. The vitamin D intake was maintained at a constant level by feeding the same amount of alfalfa hay to each cow for the duration of the lactation. The largest amount of hay that the Jersey cow would eat regularly determined the level of hay feeding for each pair. Molasses beet pulp was used to supply additional bulk to the Holstein. Vitamin D determinations were made on the alfalfa hay two or three times during the lactation period. A three-day composite sample of milk was saved each month for determining the butterfat percentage in the milk and securing a sample of pure butterfat for vitamin D assay. The milk was separated, the cream churned, and the butter melted and centrifuged to obtain the butterfat for vitamin D testing. The butterfat samples were kept frozen in sealed jars until assayed.

Because of the large amount of work involved in making the vitamin D assays it was necessary to run one pair of cows at a time. When the third pair was run blood plasma samples were taken simultaneously with the butterfat samples to assist in explaining some of the earlier observations. The regular line-test technique was used in making the vitamin D determinations on the alfalfa hay, blood plasma, and the butter-fat from the first

two pairs of cows. A modified method was used for the butterfat of the third pair to secure greater accuracy.

The modified method for vitamin D assay of low potency fats and oils. Since natural feeding stuffs were being used as the source of vitamin D in this experiment it was expected that the potency of the butterfat would be comparatively low. This raised the problem of how best to make the vitamin D determinations as rats will consume only limited amounts of the fat to be tested during the first 8 days of the usual 10-day test period. The alcoholic extraction method reported by Bechtel and Hoppert (1) was first tried but it was found that only 50–75 per cent of the vitamin D was being recovered. Further tests with modifications of this method also proved ineffective. Since Kon and Booth (3, 4) had earlier found difficulty in recovering vitamin D from butterfat either in the non-saponifiable portion or by alcoholic extraction it was decided to assay the fat direct rather than to attempt concentrating it. This method was employed for the first two pairs of cows but by that time Gridgeman, Lees, and Wilkinson (2) had reported encouraging results from adjusting the mineral content of the test diet to insure greater accuracy in the testing of low potency fats for vitamin D.

Gridgeman *et al.* (2) found a greater healing response to 0.5 International Unit of vitamin D daily with rats receiving a rachitogenic diet to which 38 per cent of an inert cooking fat had been added than in litter mates receiving the rachitogenic diet only. Their work also showed that healing responses were inversely proportional to the daily intake of salt mixture and were not related to variations in the phosphorus, protein, and fat intake. The salt mixture contained 75 per cent of $CaCO_3$, 15 per cent of NaCl, and traces of other minerals but no phosphorus. Thus the addition of fats or oils to the rachitogenic diet lowered the daily intake of salt mixture, by diluting the mineral content of the diet and by decreasing the daily consumption because of the increased caloric value of the diet. If adjustments in the salt-mixture content of the diets were made so that the intake on test diets containing fat compared favorably with the intake on the rachitogenic diet alone, the healing responses to a given amount of vitamin D were more nearly equal. With these principles in mind, studies were made of adjustments in the mineral content of rachitogenic diets used for testing low potency fats and oils for vitamin D.

In our laboratory 12 grams of butterfat had been mixed with 28 grams of the Steenbock rachitogenic diet No. 2965 to make a total of 40 grams of diet. This was consumed in about the first 8 days of the test period. The regular rachitogenic diet was then fed for the remainder of the 10-day test period. Inspection of our records showed that litter-mate rats receiving the rachitogenic diet plus the International Vitamin D Standard consumed 55 grams during the first 8 days. Adjustments were then made so that

40 grams of test diet containing the fat had the same salt-mixture content as the 55 grams of regular rachitogenic diet. On this basis each test diet was made up as follows:

17.80 grams yellow corn meal
12.00 grams fat or oil
8.00 grams wheat gluten
1.65 grams $CaCO_3$
0.55 grams NaCl

40.00 grams

For purposes of discussion this modified diet will be referred to as the mineral-adjusted diet to differentiate it from the regular rachitogenic diet. Tests were first made to determine how much healing, if any, would be initiated by feeding 12 grams of an inert fat or oil using the regular line test procedure the same as though a vitamin D assay were being made. The mineral-adjusted diet was compared with the regular rachitogenic diet and with the healing given by 5 International Units of vitamin D Standard. Ten litter-mate rats were used for each comparison. Studies were made using a sample of butterfat from a vitamin-D-deficient cow, a cooking fat, olive oil, and a cooking oil. The results are show in table 1.

TABLE 1

A comparison of the healing responses to 12 grams of inert fats and oils fed with regular rickets diet and with a mineral-adjusted diet using the regular line-test technique for vitamin D assays

Fat or oil tested	Healing responses with different diets		
	Rachitogenic only plus 5 International Units of vitamin D	Regular rachitogenic diet plus 12 grams of fat	Mineral-adjusted diet plus 12 grams of fat
Butterfat*	1.15†	0.07	0.06
Cooking fat	1.93	0.86	0.14
Olive oil	0.72	0.26	0.03
Cooking oil	1.14	0.10	0.06

* This butterfat sample was from a vitamin-D-deficient cow and contained little or no vitamin D.

† A narrow, continuous line of healing is given a value of 1.0.

It can be seen from table 1 that there was some healing response with all of the supposedly inert materials tested even with the mineral-adjusted diet but it was considerably higher when these same fats and oils were fed with the regular rachitogenic diet. The healing responses obtained from 5 International Units of vitamin D Standard gives a basis for evaluating the healing initiated by the fats alone.

In a further study, 5 International Units of vitamin D Standard were fed to ten litter-mate rats receiving 12 grams of the inert fat plus 28 grams of either the regular rachitogenic diet or the mineral-adjusted diet. These

TABLE 2

The apparent value of 5 International Units of vitamin D Standard when fed with diets containing 12 grams of inert fat mixed with regular rachitogenic diets and with mineral-adjusted diets using the line-test technique

Fat or oil tested	Apparent value of 5 I.U.† of vitamin D when fed with	
	12 grams of fat plus regular rachitogenic diet	12 grams of fat plus mineral-adjusted diet
Butterfat*	6.06	5.00
Cooking fat	7.35	5.74
Olive oil	5.41	4.10
Cooking oil	5.57	4.72
Average	6.10	4.89

* This butterfat sample was from a vitamin-D-deficient cow and contained little or no vitamin D.

† I.U. = International Unit of vitamin D.

healing responses were evaluated against litter-mate rats receiving 5 International Units of vitamin D reference oil as is used in making a regular vitamin D assay. The purpose was to determine how much, if any, the value of a known amount of vitamin D was enhanced by feeding it with the fat-containing diets. The results are shown in table 2.

It can be seen from table 2 that the apparent value of 5 International Units of vitamin D was increased in every case when fed with an inert fat added to the regular rachitogenic diet. When the mineral-adjusted diet was used the apparent value was sometimes higher than the 5 units actually fed and sometimes lower with an average of just slightly less than 5.

One other piece of evidence was obtained which gave further support to the superiority of the mineral-adjusted diet under the conditions of these determinations. Vitamin D assays were made on ten samples of butterfat using both the mineral-adjusted diet and the regular rachitogenic diet. The usual line-test technique was followed. Litter-mate rats were given 2 units

TABLE 3

A comparison of the vitamin D in ten samples of butterfat as determined by using the regular rachitogenic diet and the mineral-adjusted diet

Number of butterfat sample	I.U.* of vitamin D per gram of butterfat			
	Cow 13E		Cow 150	
	Rachitogenic diet	Mineral-adjusted diet	Rachitogenic diet	Mineral-adjusted diet
1	0.33	0.23	0.68	0.35
2	0.40	0.25	0.50	0.30
3	0.22	0.19	0.37	0.32
4	0.22	0.20	0.38	0.31
5	0.27	0.20	0.28	0.22
Average	0.29	0.21	0.44	0.30

* I.U. = International Unit of vitamin D.

and 5 units, respectively, of International Standard vitamin D and the final potencies calculated by the use of a master curve of response to graded doses of vitamin D. The results are shown in table 3.

In every case the value was higher with the regular rachitogenic diet than when the mineral-adjusted diet was employed. The average difference was 38 per cent higher for one cow and 47 for the other. With more potent butterfat the percentage differences would probably not be so great.

While we still do not have a satisfactory method for concentrating the vitamin D of low potency butterfat samples, the combined evidence from the work just presented indicates that the mineral-adjusted diet as used under the conditions of this experiment gave more accurate results than where the regular rachitogenic diet was employed. Consequently the vitamin D values for the butterfat were determined for the third pair of cows in this experiment by using the mineral-adjusted diet technique. Just what adjustments would be necessary for different doses of butterfat require further investigation.

RESULTS AND DISCUSSION

The pertinent data from the first pair of cows are shown in table 4.

These two animals were typical of their respective breeds. The Holstein gave between two and three times as much milk as the Jersey but the butterfat percentage was only about half as high. As a result the total butterfat production was slightly higher for the Holstein. Each cow received 19,000 International Units of vitamin D daily throughout the lactation in the alfalfa hay fed. Under these conditions the butterfat from the Jersey averaged about 50 per cent higher in International Units of vitamin D per gram. The higher butterfat content of Jersey milk and the higher vitamin D potency per gram made an average of approximately 30 International Units of vitamin D per quart of milk whereas the Holstein milk averaged only about 10 units per quart. Because of the higher milk production of the Holstein the total amount of vitamin D recovered daily in the milk was not greatly different for the two cows. The recovery for the two cows amounted to 1.50 to 1.75 per cent of the intake during the flush of the lactation and declined to 0.50 to 0.75 per cent toward the end. The percentage of recovery of ingested vitamin D in the butterfat, and the greater efficiency of recovery at higher levels of production, agree in general with the findings of other investigators. It is interesting to note that the vitamin D potency per gram of butterfat decreased with the decrease in milk production near the end of the lactation but the percentage of butterfat in the milk increased proportionately, so the potency per quart of milk remained quite constant throughout the lactation.

In order to conserve space the data for the second pair of cows will not be published. The general relationships were essentially the same as for

the first pair. The alfalfa hay fed to the second pair contained less vitamin D so each cow received only 6,500 International Units daily. This resulted in a lower vitamin D potency per gram of butterfat and per quart of milk. The Holstein milk averaged about 6 International Units per quart and the Jersey about 15.

TABLE 4

A comparison of the vitamin D content of the milk and butterfat produced by a Jersey and a Holstein cow receiving 19,000 International Units of vitamin D daily

First pair

Month of lactation	Daily production			Vitamin D			
	Milk, lbs.	Fat, %	Fat, gms.	I.U.* per gm. of fat	I.U. per qt. of milk	Recovered in fat	
						Daily I.U.	% of intake
Holstein cow—390							
I	45.6	3.85	793.8	0.31	11.6	246	1.09
II	55.5	3.82	961.6	0.37	13.8	356	1.86
III	58.0	3.17	830.1	0.40	12.3	332	1.74
IV	54.7	2.81	698.5	0.43	11.8	300	1.75
V	41.9	2.06	390.1	0.45	9.0	176	1.06
VI	32.2	3.77	548.9	0.29	10.6	159	0.90
VII	32.4	3.60	526.2	0.25	8.8	132	0.69
VIII	32.4	3.30	485.4	0.25	8.0	121	0.63
IX	32.1	3.35	485.4	0.27	8.8	131	0.69
X	35.4	3.20	512.6	0.23	7.2	118	0.62
XI	33.7	3.49	535.2	0.27	9.2	145	0.76
XII	28.2	3.95	503.5	0.26	10.0	131	0.69
XIII	15.5	4.27	299.4	0.30	12.5	90	0.47
Jersey cow—146							
I	17.0	5.81	449.1	0.28	15.9	126	0.60
II	16.0	6.09	444.5	0.53	31.6	236	1.23
III	16.8	5.40	412.8	0.61	32.3	252	1.43
IV	15.6	5.19	367.4	0.63	31.8	231	1.47
V	15.3	5.45	376.5	0.63	33.3	237	1.40
VI	13.3	6.11	367.4	0.42	24.9	154	0.83
VII	12.9	6.40	376.5	0.43	27.0	162	0.85
VIII	12.1	6.60	358.3	0.49	31.3	176	0.92
IX	7.8	7.20	254.0	0.48	33.6	122	0.64
X	7.4	7.10	235.9	0.44	30.3	104	0.55
XI	6.3	8.00	226.8	0.43	33.6	98	0.51

* I.U. = International Unit of vitamin D.

For the third pair of cows the vitamin D content of the blood plasma was determined simultaneously with the monthly butterfat samples and the mineral-adjusted diet was used for the vitamin D determinations on the butterfat. The data for the third pair of cows are shown in table 5.

The general relationships discussed for the first pair of cows also hold true for the data from the third pair. Although these cows received 24,200 International Units of vitamin D daily which is slightly more than the first pair the potency of the butterfat per gram is slightly less. The fact that

these assays were made with the mineral-adjusted diet which gives somewhat lower but more accurate results undoubtedly explains this difference. Again, the Jersey butterfat has a higher vitamin D potency per gram than the Holstein. The potency of the blood plasma is also higher for the Jersey even though the vitamin D intake was kept the same for both cows starting at two months before calving. The higher potency of blood plasma undoubtedly explains why the Jersey butterfat had a higher vitamin D potency

TABLE 5

A comparison of the vitamin D content of the blood plasma and of the milk and butterfat produced by a Jersey and a Holstein cow receiving 24,200 International Units of vitamin D daily

Third pair

Month of lactation	Daily production			Vitamin D				
	Milk, lbs.	Butterfat, %	Butterfat, gms.	I.U.† per ml. of blood plasma	I.U. per gm. of butterfat	I.U. per qt. of milk	Recovered in fat	
							Daily I.U.	% of intake
Holstein Cow—13E*								
I	48.8	3.9	861.0	2.33	0.24	9.12	206.8	0.855
II	41.0	1.6	299.4	2.53	0.25	3.91	74.9	0.310
III	39.8	3.8	685.0	3.34	0.19	7.03	130.2	0.538
IV	37.0	4.0	671.0	3.21	0.20	7.78	134.2	0.555
V	38.3	3.6	626.0	3.57	0.20	7.01	125.2	0.517
VI	32.8	3.8	567.0	3.08	0.19	7.05	107.7	0.445
VII	36.0	3.4	553.4	2.49	0.20	6.60	110.7	0.457
Jersey cow—150								
I	20.2	5.3	485.4	3.71	0.42	21.69	203.9	0.843
II	18.3	5.7	473.1	3.47	0.30	16.66	141.9	0.586
III	20.8	5.7	537.5	4.70	0.32	17.76	172.0	0.711
IV	16.2	6.0	440.9	3.93	0.31	18.15	136.7	0.565
V	19.7	5.6	500.3	4.39	0.22	12.02	110.1	0.455
VI	18.8	6.0	511.7	3.58	0.29	16.96	148.4	0.613
VII	18.0	6.2	506.2	2.25	0.21	12.70	106.3	0.439
VIII	18.8	5.6	477.6	3.00	0.23	12.56	109.8	0.454
IX	18.0	6.2	506.2	2.87	0.23	13.91	116.4	0.481
X	16.3	6.4	473.1	2.85	0.24	14.96	113.5	0.469

* Lactation incomplete—cow sold as T.B. reactor at end of seventh month.
† I.U. International Unit of vitamin D.

than the Holstein in each of the three pairs of cows used in this experiment. Just why the blood plasma of the Jersey should be higher on the same intake of vitamin D still remains unexplained.

Light, Wilson, and Frey (6) found a direct correlation between the concentration of vitamin D in the blood and the rate of secretion into the milk when cows were fed irradiated yeast. The results of the present investigation show that this same relationship exists when natural feeds alone furnish a much smaller intake of vitamin D. Higher blood plasma potencies are

associated with higher butterfat potencies and lower blood plasma values with lower butterfat values.

It should be noted that in this experiment the Holstein and Jersey cow making up each pair were given the same vitamin D intake. The relationships found will properly apply only where these conditions prevail. Under normal herd management Holstein cows will consume more roughage and hence have a higher vitamin D intake than Jerseys. This would undoubtedly increase the vitamin D potency of the butterfat produced by the Holstein, making it more nearly that of the Jersey. Just what these relationships would be cannot be told without further experimentation. It would also require further investigation to ascertain the breed effect of exposure to sunshine which is ordinarily experienced in normal herd management.

SUMMARY

Breed differences in the vitamin D content of milk and butterfat for Holstein and Jersey cows receiving the same vitamin D intake have been investigated. Three comparable pairs of animals have been studied for complete lactation periods. The first pair received 19,000 International Units of vitamin D daily in the alfalfa hay which was the only source of this factor. The second pair received 6,500 units daily and the third pair 24,200. The milk from each cow was saved for a three-day period once each month for determining the percentage of butterfat in the milk and to secure a sample of pure butterfat for vitamin D determination. Blood plasma samples for vitamin D determinations were taken simultaneously with the butterfat samples for the third pair of cows.

The results from all three pairs are in general agreement in showing that under the conditions of this experiment the Jersey butterfat contained somewhat more vitamin D per gram of fat than the Holstein. The higher butterfat percentage for the Jersey milk combined with a higher vitamin D potency per gram of fat made the Jersey milk two to three times more potent than the Holstein milk in vitamin D per quart. The total amount recovered in the butterfat daily was approximately the same for both breeds, however, because of the larger milk production of the Holstein. About 1.50 per cent of the ingested vitamin D was recovered in the butterfat during the flush of the lactation but only about 0.50 per cent near the end of the lactation. The vitamin D potency per gram of butterfat decreased for each breed as the lactation progressed but the butterfat percentage increased proportionately so the vitamin D content per quart of milk remained quite uniform for each cow throughout the lactation period. Data from the third pair of cows indicated that the vitamin D content of the blood plasma was higher for the Jersey. This correlated with the higher potency of the butterfat.

A modification in the mineral content of the rachitogenic diet used for making vitamin D assays has been developed which under the conditions of

this experiment gave lower but more accurate values for the vitamin D content of low-potency butterfat samples.

REFERENCES

(1) Bechtel, H. Ernest, and Hoppert, C. A. A Study of the Seasonal Variation of Vitamin D in Normal Cow's Milk. Jour. Nutr., 11, No. 6: 537–549. 1936.

(2) Gridgeman, N. T., Lees, H., and Wilkinson, H. The Biological Vitamin D assay of Low Potency Materials with Special Reference to the Role of the Mineral Content of the Diet. Biochem. Jour., 33, No. 5: 645–654. 1939.

(3) Kon, S. K., and Booth, R. G. The Vitamin D Activity of Butter. I. A Chemical Differentiation of the Antirachitic Factor of Autumn and Winter Butter from Irradiated Ergosterol and the Vitamin D of Cod Liver Oil. Biochem. Jour., 27, No. 4: 1302–1309. 1933.

(4) Kon, S. K., and Booth, R. G. The Vitamin D Activity of Butter. II. The Complex Nature of the Antirachitic Action of Butter. Biochem. Jour., 28, 111–120. 1934.

(5) Kon, S. K., and Henry, K. M. A Comparison of the Vitamin D Contents of Guernsey and Shorthorn Butter (Milk). Biochem. Jour., 30, No. 5: 776–779. 1936.

(6) Light, R. F., Wilson, L. T., and Frey, C. N. Vitamin D in the Blood and Milk of Cows Fed Irradiated Yeast. Jour. Nutr., 8, No. 1: 105–111. 1934.

(7) Standard Brands, Inc. The Natural Production of Metabolized Vitamin D Milk. Bulletin, Standard Brands Inc., 595 Madison Ave., New York, N. Y.

LACTIC ACID IN DAIRY PRODUCTS. I. APPLICATION OF THE HILLIG METHOD*

I. A. GOULD

Department of Dairying, Michigan Agricultural Experiment Station, East Lansing, Michigan

Quantitative determinations of lactic acid and lactates in milk and milk products as means of ascertaining the extent of microbiological spoilage have long been of interest and various methods have been proposed for such analyses. Recently, an intensification of interest in this question has resulted because of the use of lactic acid values by Federal regulatory officials as a basis for judging quality in concentrated and dried milk.

At the present time there are two rather generally accepted quantitative methods for measuring lactic acid in milk: (a) The Troy-Sharp aldehyde procedure (7) and (b) the Hillig colorimetric procedure (1).

The aldehyde procedure involves the precipitation of the interfering substances with copper hydroxide at 45° C., oxidizing the filtrate under carefully controlled conditions with sulfuric acid, manganese sulfate and potassium permanganate, distilling the acetaldehyde produced into sodium bisulfite, and titrating the bound sulfite with iodine. The result is then corrected for the blank on fresh milk and for the lactic acid retained by the precipitate. This method was found by Troy and Sharp to be accurate down to 0.002 per cent of lactic acid in the original milk and to give excellent agreement in the hands of various operators when applied to dried milk.

The colorimetric method utilizes the following steps: (a) Preparation of a milk serum by treatment of the milk with sulfuric and phosphotungstic acids, (b) extraction of the serum with ethyl ether, (c) neutralization of the extract with barium hydroxide and the subsequent precipitation and removal of the alcohol-insoluble barium salts, (d) treatment of the filtrate with carbon and hydrochloric acid, (e) photometric measurement of the intensity of the yellow color produced upon the addition of ferric chloride.

The accuracy of the colorimetric method was determined by Hillig (1) by adding lithium lactate to reconstituted dried skim milk at concentrations varying from 0.002 to 0.150 per cent. Recovery was usually within one milligram per cent.

Limited comparisons of the aldehyde and colorimetric methods have been made by Hillig (2). On two samples of dried skim milk which were sent to collaborators, the Troy-Sharp procedure gave fairly satisfactory results, but results by the colorimetric method were unsatisfactory. The failure of the colorimetric procedure to give uniform results was believed to be due to

Received for publication February 7, 1944.

* Journal Article No. 683, new series, Michigan Agricultural Experiment Station.

inaccurate standard curves. In another study, Hillig (3) found good agreement between the two methods when they were applied to non-neutralized dry milk, but the aldehyde method gave somewhat lower results on neutralized samples. In more recent work, Hillig (5) proposes an improvement in the procedure for establishing the standard curve for the colorimetric determination which presumably insures greater uniformity in the application of the method.

Until quite recently, the aldehyde technique has been more widely used than the colorimetric method, probably because the former was perfected at an earlier date and more is known concerning the factors which influence the reliability of its results. Troy and Sharp (7) found that titers obtained by the aldehyde method were markedly increased by lipolysis (due to the presence of glycerol), and by such preservatives as formalin and acetone. Slight increases were produced by the use of phenol and salicylic acid, whereas potassium dichromate was reported to decrease the lactic acid recovery. Ethyl and methyl alcohol in amounts of one to two per cent did not interfere with the determination. Products high in sucrose could not be successfully analyzed by the aldehyde method.

These workers also reported mercuric chloride in concentrations of 0.2–0.5 per cent to be a satisfactory preservative for as long as a month for milk kept in dark cool surroundings. Later observations (8) revealed increases in the lactic acid of milk stored in diffused sunlight and preserved with mercuric chloride, increases which were attributed to proteolysis and the formation of degradation products which interfered with the acetaldehyde titration. Heating of the samples to 80° C. for five minutes decreased but did not stop the proteolysis.

At the present time the colorimetric procedure is receiving considerable attention since this technique is being used by the Federal regulatory officials in the examination of milk and other food products. However, information is limited relative to the factors which may influence the reliability of the results secured by this procedure. It was for the purpose of securing such information that the study herein reported was conducted.

PROCEDURE

Lactic acid was measured by the Hillig colorimetric method (1) with slight modifications. The calcium lactate-barium hydroxide procedure was used for the standard curve (5).

Colorimetric measurements were made by means of a Cenco-Sheard Spectrophotelometer using tubular cells, a wave-length setting of 400 millimicrons, and a blue filter over the entrance slit to reduce stray light. The use of this wave length was established from a transmission curve developed by using the Spectrophotelometer and an aqueous solution containing approximately 12 mg. of lactic acid (fig. 1). The curve is similar to that

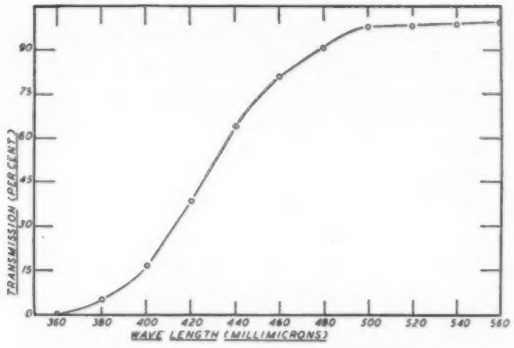


FIG. 1. Transmission curve for a solution of known lactic acid content.

reported by Hillig (1) but indicates somewhat more absorption at the lower wave length values. At 400 millimicrons wave length, the transmission is approximately 19 per cent, which corresponds to approximately the result found by Hillig at 460 millimicrons wave length.

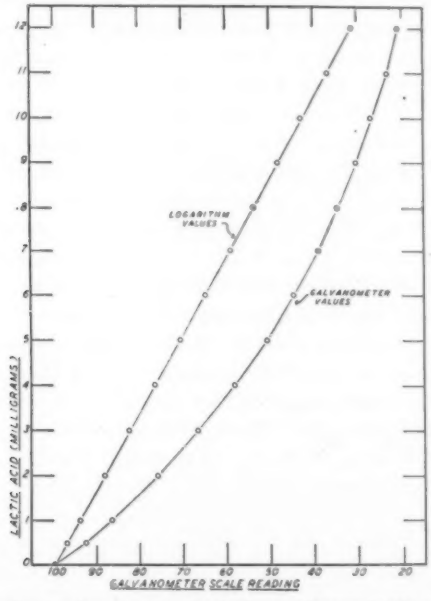


FIG. 2. Standard curve from prepared solutions of known lactic acid content. (Lactic acid concentration plotted directly against galvanometer readings and also against the logarithms of the galvanometer readings. For convenience, the characteristic of the logarithm, *i.e.*, 1 or 2, has been omitted.)

A standard curve was prepared according to the direction of Hillig (5). Curves based both on the direct galvanometer readings and on the logarithm of the galvanometer readings are presented in figure 2. The logarithm values exhibit almost a straight line relationship with the lactic acid concentration, and for this reason were used throughout this study for determining the lactic acid concentrations of the unknowns.

Precautions suggested by Hillig (1), to prevent fading of the color following the addition of the ferric chloride were found unnecessary. Samples were treated with ferric chloride and the color determinations made within 2–3 minutes thereafter. Numerous trials indicated that exposure to diffused daylight for approximately 10 minutes or longer was necessary before any detectable color fading occurred.

Special precautions were found to be necessary to insure properly purified ether for the extraction. Ether of U.S.P. grade was found to vary in its ability to extract lactic acid, but in general, was highly inefficient. In some trials, such ether recovered only approximately 30 per cent of the lactic acid, and frequently less than 50 per cent of the acid was recovered. Satisfactory results were secured when the ether was shaken vigorously with water (four parts ether to one part ordinary tap water) and then distilled just before use. Attempts to substitute Skellysolve A (a petroleum product with a boiling point similar to ether) as the extractant were unsuccessful.

RESULTS

Recovery of added acid. The accuracy of the Hillig method was ascertained by analyzing milk to which different concentrations of lactic acid had been added. The results of one series of such trials are shown in table 1.

These data indicate that the method possesses a high degree of accuracy and reliability. Recovery of acid was within 1 mg. for all but one sample and the percentage recovery was usually well within 5 per cent. The average recovery for the 14 analyses was 99.7 per cent.

Lactic acid values of fresh milk. Milk samples secured from the afternoon milking of cows of the Michigan State College herd were immediately placed in ice water, transported to the laboratory and analyzed for lactic acid by the Hillig technique. The results, presented in table 2, reveal a range in lactic acid from 0.92 mg. to 3.02 mg. per 100 gms. for the six samples, with an average of 1.93 mg. per 100 gms. These are similar to the results given for fresh milk by Troy and Sharp (6) and for commercial, retail samples by Hillig (1). In general, it appears that the lactic acid content of fresh milk, determined by this method, will usually not exceed 3 mg. per cent, but factors of season, disease and stage of lactation or gestation may influence this result.

Even though the data in table 2 indicate the presence of small amounts of lactic acid in strictly fresh milk, it should be realized that these results

TABLE 1

Recovery of known quantities of lactic acid added to milk

Sample No.	Duplicate	Acid added	Acid recovered	Difference	Recovery
		mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*per cent*
1	a	1.79	1.55	−0.24	86.6
	b	1.79	1.78	−0.01	99.4
2	a	8.97	9.78	+0.81	109.0
	b	8.12	8.58	+0.46	105.7
3	a	17.95	18.07	+0.12	100.7
	b	17.95	17.43	−0.52	97.1
4	a	35.36	34.54	−0.82	97.7
	b	35.90	35.40	−0.50	98.6
5	a	53.04	52.32	−0.72	98.6
	b	53.04	52.44	−0.60	98.9
6	a	70.72	71.68	+0.96	101.4
	b	70.72	72.98	+2.26	103.2
7	a	106.08	105.69	−0.39	99.6
	b	106.08	105.13	−0.95	99.1

may be due to constituents other than lactic acid. In fact, for the present, it is probably more logical to accept these results as representing the residual or blank values of fresh milk rather than as indicating lactic acid. Furthermore, since results obtained by the Hillig (or similar) method are due either entirely or partly to this residual factor (unless a blank correction is made for the milk), they may not, in a strict sense, represent the true lactic acid content. On this basis, therefore, it appears logical to refer to the results of such an analysis as "lactic acid values." This practice is adhered to throughout this study.

Preservatives. To determine their influence on the lactic acid values, the following preservatives were added to milk: Mercuric chloride (commercial corrosive sublimate), formalin, potassium dichromate, boric acid, sodium benzoate and phenol.

Following the addition of the preservative, each sample was held at 37° C. for several hours until its control sample was strongly sour to the taste. Lactic acid was determined on the milk immediately following the addition of the preservative and again at the close of the storage period.

The results of this experiment, presented in table 3, reveal that with the exception of potassium dichromate and sodium benzoate, the lactic value

TABLE 2

Lactic acid values of fresh milk as determined by the Hillig procedure

Cow No.	Breed	Lactic acid content
		mg./100 gm.
9	Guernsey	1.6
22	Guernsey	2.0
114	Jersey	2.9
274	Holstein	0.9
302	Brown Swiss	3.0
405	Holstein	1.3

TABLE 3

Influence of preservatives on the lactic acid value of fresh milk and their effectiveness of preservation

Preservative	Concentration of preservative	Lactic acid value	
		Before storage	After storage
	per cent	*mg./100 gm.*	*mg./100 gm.*
Control	0.00	1.9	19.8
Mercuric chloride*	0.24	2.2	1.7
Formalin	0.10	1.7	1.9
Control	0.00	3.5	31.7
Potassium dichromate	0.50	2.1	1.7
Boric acid	0.75	3.5	3.2
Control	0.00	2.2	15.8
Sodium benzoate	0.75	21.1	26.8
Phenol	0.20	2.1	4.6

* Commercial corrosive sublimate—true mercuric chloride concentration considerably less.

of fresh milk is essentially unaffected by the preservatives tested. The concentration of dichromate was considerably higher than is generally used for preservation of milk, which may account for the lower result for lactic acid obtained when this preservative was used. Sodium benzoate apparently increased greatly the lactic acid value of the milk and therefore appears to be an unsatisfactory preservative for samples which are to be analyzed by this technique.

These data also indicate that, with the concentrations used, mercuric chloride, formaldehyde, potassium dichromate, and boric acid prevented lactic acid development during storage of the milk, whereas sodium benzoate and phenol were less effective. In one series, the mercuric chloride and formaldehyde-containing samples (following their storage at 37° C. for several hours) were held at 5° C. for 12 days. The lactic acid content did not increase during this period of time.

TABLE 4

Influence of lipolysis on the lactic acid content of milk

Sample	Lactic acid value	
	Before storage	After storage
	mg./100 gm.	*mg./100 gm.*
Series I		
Sample 1	2.3	2.5
Sample 2	1.7	2.6
Sample 3	2.0	2.3
Series II		
Sample 1	3.3	3.2
Sample 2	3.2	3.0
Sample 3	2.7	3.0

Influence of lipolysis. The effect of the development of rancidity on the lactic acid determination was determined by resorting to two somewhat different procedures. In one procedure (Series I), three different samples of raw milk were mixed in equal proportion with pasteurized, homogenized milk. This procedure was shown previously to produce rancidity within a few hours (6). Formalin was added at the rate of 0.1 per cent to prevent bacterial activity. Lactic acid measurements were made immediately after mixing the non-homogenized and homogenized portions and again after the mixtures had been held at 4° C. for approximately 6 hours.

In another procedure (Series II) three samples of raw milk were homogenized at 2500 pounds pressure and stored for 48 hours at 4° C. Formalin was added as in Series I to prevent bacterial spoilage. The lactic acid content of the milk was measured at the beginning and after the 48-hour storage.

The results of the lipolysis studies are presented in table 4.

TABLE 5

*Influence of ethyl alcohol in the extracting ether or in the milk on lactic acid recovery**

Concentration of alcohol		Lactic acid value of milk when alcohol added to	
In ether	In milk	Ether	Milk
per cent	*per cent*	*mg./100 gm.*	*mg./100 gm.*
0	0	41.4	10.3
3	1	44.8	10.1
6	2	44.8	10.0

* Lactic acid was added to milk at rate of 39 mg./100 gm. in the ether trials and at rate of 9 mg./100 gm. in the milk trials.

In general, the data in table 4 reveal no appreciable influence of fat hydrolysis on the lactic acid determination. In other experiments, as much as 2 per cent glycerol was added to milk without altering the lactic acid results.

Influence of ethyl alcohol in ether and in milk. Since observations had revealed that the use of impure ether (U.S.P. grade) for extraction resulted in appreciably reduced recovery of added lactic acid, trials were conducted to determine if the presence of alcohol in the ether was the vitiating factor. Also, a study was made of the recovery of lactic acid from milk to which both lactic acid and ethyl alcohol had been added.

The results are presented in table 5.

The results show that the addition of either 3 or 6 per cent alcohol to the ether used for extraction resulted in an increase of approximately 8.2 per cent in the lactic acid recovered. This would indicate that the low recovery of lactic acid when impure ether is used is not due to the presence of ethyl alcohol in the ether.

TABLE 6

Influence of the addition of salts or sucrose upon the lactic acid content of milk

Sample	Amount added	Lactic acid content
	per cent	*mg./100 gm.*
Control	0.00	2.5
Sodium chloride	0.25	2.5
Sodium chloride	0.50	2.9
Control	0.00	2.4
Sodium citrate	0.20	2.2
Disodium phosphate	0.20	2.4
Control	0.00	2.6
Sucrose	5.00	2.3
Sucrose	10.00	2.9
Control*	0.00	22.6
Sucrose*	5.00	22.8
Sucrose*	10.00	22.4

* Lactic acid added to milk samples.

The results in table 5 also reveal that the addition of ethyl alcohol to milk in concentrations of 1 or 2 per cent did not appreciably affect the lactic acid recovery.

Influence of salts and sugar. Trials were conducted in which sodium chloride, sodium citrate, disodium phosphate and sucrose were added to milk. The lactic acid values for these trials are presented in table 6. The results reveal that these compounds, in the concentrations used, have no influence on the lactic acid values as determined by the Hillig procedure.

Influence of neutralization. Milk was permitted to undergo appreciable souring and was then divided into two lots. One lot was neutralized either with sodium carbonate or sodium bicarbonate to approximately the original pH and the second was neutralized to somewhat over pH 7. The results are presented in table 7.

These data indicate that neutralization of milk with sodium bicarbonate or sodium carbonate slightly increases the lactic acid values as measured

TABLE 7

Influence of neutralization on the lactic acid values of milk

Sample	pH	Lactic acid values
		mg./100 gm.
Neutralizer—$NaHCO_3$		
Fresh milk	6.58	.....
Soured milk	6.32	57.9
Neutralized	6.68	62.6
Overneutralized	7.25	65.8
Neutralizer—Na_2CO_3		
Fresh milk	6.56	.....
Soured milk	6.29	53.2
Neutralized	6.59	55.2
Overneutralized	7.27	58.4

by the Hillig technique. Similar results have been obtained when neutralization was with disodium phosphate.

DISCUSSION

Results secured in this study indicate that the Hillig colorimetric method for determining lactic acid in milk is reliable and accurate when applied to milk containing up to approximately 100 mg. per cent of lactic acid, thus agreeing closely with the results presented by Hillig (1). On this basis the method appears to be a definite contribution to the determination of quality in dairy products.

Application of this test (or a test of similar accuracy) to quality control by state and municipal regulatory officials would be highly desirable. However, the time and skill required for making the determination may limit its general application. Approximately eight hours are necessary for making an analysis, about one-half of this being utilized for ether extraction of the serum. The time disadvantage may be overcome somewhat by having a series of extractors operating simultaneously.

One of the major advantages of the colorimetric method is its constancy. Several factors which have been reported by Troy and Sharp (7) to influence the aldehyde method were without effect upon the colorimetric method. Thus, the method may be utilized in analyzing (a) products high in sugar, (b) products which have undergone considerable lipolysis, and (c) milk products which have been preserved for long periods with mercuric chloride. The accuracy of the method for preserved samples is of especial value to a laboratory relying upon preservation of field samples.

Another desirable feature of the colorimetric method is the elimination of a titration procedure. This is of advantage in a laboratory doing routine analyses where, once the procedure is standardized, considerable technical skill is not required to conduct the test. However, this presupposes the use of a photoelectric colorimeter for measuring color intensity. In fact, the use of a photometer rather than an ordinary visual colorimeter or Nessler tubes would appear to be essential if a high degree of accuracy is to be obtained with low concentrations of lactic acid, since a visual matching of the yellow color produced is not easy.

SUMMARY AND CONCLUSIONS

The Hillig colorimetric lactic acid determination usually gave recovery of lactic acid from milk within 0.001 per cent. Freshly drawn milk was found to give values equivalent to 0.9 to 3.0 mg. lactic acid per 100 grams milk by this method. These values probably represent the blank for strictly fresh milk rather than lactic acid.

The colorimetric method was unaffected by the concentrations of mercuric chloride, formalin, boric acid and phenol employed in this study; by

development of rancidity; or by the addition to the milk of certain concentrations of sodium chloride, sodium citrate, disodium phosphate, sucrose, and ethyl alcohol. Sodium benzoate markedly increased the lactic acid value and potassium dichromate appeared to have a slight depressing effect.

Neutralization appears to slightly increase the lactic acid values obtained by this method.

REFERENCES

(1) HILLIG, F. The Colorimetric Determination of Lactic Acid in Milk and Milk Products. Jour. Assoc. Off. Agr. Chem., 20: 130–141. 1937.

(2) HILLIG, F. Report on Neutralizers in Dairy Products. Jour. Assoc. Off. Agr. Chem., 22: 495–496. 1939.

(3) HILLIG, F. Report on Dried Milk. Jour. Assoc. Off. Agr. Chem., 24: 548–550. 1941.

(4) HILLIG, F. Effect of Neutralization on Sour Milk in the Manufacture of Dried Skim Milk. Jour. Assoc. Off. Agr. Chem., 25: 253–264. 1942.

(5) HILLIG, F. Colorimetric Determination of Lactic Acid. Jour. Assoc. Off. Agr. Chem., 26: 199. 1943.

(6) LARSON, P. B., TROUT, G. M., AND GOULD, I. A. Rancidity Studies on Mixtures of Raw and Pasteurized Homogenized Milk. JOUR. DAIRY SCI., 24: 771–778. 1941.

(7) TROY, H. C., AND SHARP, P. F. Quantitative Determination of Lactic Acid in Dairy Products. Cornell Agr. Exp. Sta. Memoir 179, 49 pp., illus. 1935.

(8) TROY, H. C., AND SHARP, P. F. Mercuric Chloride as a Preservative for Milk Samples Held for the Determination of Lactic Acid. JOUR. DAIRY SCI., 20: 77–81. 1937.

LACTIC ACID IN DAIRY PRODUCTS. II. RELATION TO FLAVOR, ACIDITY MEASUREMENTS, BACTERIAL COUNT AND METHYLENE BLUE REDUCTION*

I. A. GOULD AND J. M. JENSEN

Department of Dairying, Michigan State College, East Lansing, Michigan

The lactic acid content of concentrated milk products reflects the quality of the raw milk used in their manufacture, and application of lactic acid determinations is now being made with this in mind. The present problem of the manufacturers of evaporated, condensed and dried milk is to grade the raw milk supply so that the final product will not contain an excessive amount of lactic acid. To accomplish this, use of lactic acid measurements on the raw milk would be the ideal procedure, but this is impractical because of the time and skill required for the determination. However, the plant operator may establish standards with more practical grading methods that will insure low lactic acid values in the manufactured products.

Practical methods commonly used for the grading of the raw milk supply include (a) the direct microscopic test, (b) the methylene blue reduction test, (c) the organoleptic test, *i.e.*, odor and taste, and (d) titrable acidity. Much information is available relative to the use of these procedures to determine quality in milk, but their applicability to a grading program having a lactic acid basis has been incompletely ascertained. It was for the purpose of securing more information along this line that the investigation reported in this paper was conducted.

Various studies have been conducted to determine the relationship between acidity measurements and acid flavor. As early as 1891, Stokes (14) found milk containing various preservatives to taste sour when the titrable acidity was from 0.4 to 0.49 per cent, whereas unpreserved milk usually tasted sour when the acidity reached 0.3 per cent. Richmond and Harrison (8) in 1900 observed milk to taste sour at an acidity of 0.405 per cent. Later (1918) Van Slyke and Baker (16) made observations at frequent intervals of milk undergoing souring and, when the first perceptible characteristic sour milk flavor was detected, determined the pH and titrable acidity. The results on seven samples indicated that appreciable acidity had developed in the majority of the samples by the time the flavor was detected, with pH values ranging from 5.44 to 5.96 (decidedly below the normal pH range of 6.3–6.7) and with the titrable acidity ranging from approximately 0.33 to 0.13 per cent, expressed as lactic acid. Four of these samples were permitted to sour naturally at 25° C. At the first signs of souring the pH values ranged from 5.49 to 5.44, and the titrable acidity from approxi-

Received for publication February 7, 1944.

* Journal Article No. 284, New Series, Michigan Agricultural Experiment Station.

mately 0.22 to 0.33 per cent. These workers concluded that there was no relationship between the development of the characteristic sour-milk flavor and the pH and titrable acidity.

Hammer and Hix (5) inoculated aseptic milk with strains of *S. lactis* and determined the acidity changes and bacterial plate counts in milk when a change in flavor appeared. These workers differentiated between the "sour" flavor and the flavor distinguished "by acidity increases only" and could find no definite relationship between the development of "sour" taste and increase in acidity. In certain cases, milk showing acidity increases of 0.04–0.05 per cent was classed as "sour" by taste whereas other milk with the same acidity changes was classed as showing only a distinct rise in acidity. One sample was considered to be "sour" when the acidity had increased 0.03 per cent, and another with an acidity increase of 0.02 per cent was graded as having a distinct rise in acidity. Their results reveal, however, that when the milk exhibited either a rise in acidity by taste or a "sour" flavor, the actual titrable acidity had increased in every case, the increases varying from 0.02 to 0.08 per cent and with the majority varying from 0.04 to 0.06 per cent, expressed as lactic acid.

These workers found only a general relationship to exist between acidity increases and numbers of organisms present, but noted that large increases in bacteria had occurred by the time acidity changes were detected by taste. In one series, seven samples which exhibited definite rise in acidity gave plate counts ranging from 30 to 90 million bacteria per ml., and four "sour" samples (by taste) possessed counts of 53 to 116.5 million bacteria per ml. In another series, those samples showing a rise in acidity had bacterial counts of 37.5 to 127 million per ml., and those which were classed as sour showed bacterial counts of 91 to 235 million per ml.

Schultz, Marx and Beaver (9) determined the pH of milk colorimetrically and correlated the findings with bacterial count. The conclusions were that the bacterial count of milk begins to increase markedly at pH 6.6 (approximately 16 million per ml.) and at pH 6.5 the count reached an average of 100 million per ml. The acidity was first detected by taste at about pH 6.0. Analysis of some of the data of Cooledge and Wyant (1) reveals the milk to have a "sour" flavor at pH values ranging from 5.8 to 6.4, although the authors made no attempt to correlate the pH with the first changes in milk flavor.

Sommer and Hart (11) indicate that an increase of 0.03 per cent titrable acidity in milk represents the maximum increase permitted in grading milk, and Sommer (10) observes that acidity increases of 0.02 per cent and sometimes as high as 0.05 per cent are necessary before a "sour" odor is detectable.

Although it is often the practice to class milk flavors associated with acid development under the heading of "high-acid" or "sour," considerable

doubt has been expressed that the flavor produced is necessarily related to lactic acid formation. Van Slyke and Baker (16) refer to the first perceptible sign of souring as "a characteristic flavor, discernible to the senses of both smell and taste, due to the presence of some volatile compound formed in the souring process and not to lactic acid. The flavor appears before the milk begins to taste acid." They further indicate that the flavor is the result of bacterial action, and the compounds responsible unidentified.

Sommer (10) also points out that the "sour" odor is not due to lactic acid but to volatile substances such as acetic, propionic and formic acids, acetaldehyde, acetone, diacetyl and methylacetylcarbinol, and that the proportion of these to lactic acid governs the extent to which milk must sour before the change is detected by odor.

Another type of flavor which is closely associated with acid development is the "malty" flavor. Some milk judges class this flavor defect as a high-acid or sour-milk flavor, whereas others refer to it as a "bacterial" flavor. The flavor has been found to be produced by a streptococcus (4), by a micrococcus alone and in cooperation with a bacillus (15), and may also result through the action of lactobacilli (13).

Hammer and Cordes (4) found a streptococcus (*S. lactis* var. *maltigenes*) to produce the malty or burnt flavor, and studied the development of the flavor in sterile milk by inoculation with the causative organism. Titrable acidity values on the milk when the flavor was first observed ranged from 0.25 to 0.38 per cent. They stated that "it is evident that the caramel (or malty) flavor developed at a low acidity, although the flavor was never noticed until there had been a definite increase in acid." Hammer (3) later stated that, "the organisms can produce a malty condition with no significant increase in acidity, and sweet milk or cream is sometimes distinctly malty, although sour products are more commonly involved."

The maltiness produced by micrococcus was found by Tracy and Ramsey (15) to be intensified when a spore-forming rod was present. The flavor was found in milk in which the titrable acidity was usually not above 0.18 per cent. In two trials with methylene blue, malt-flavored milk with a bacterial count of 700,000 per ml. reduced the dye in 2.25 hours in one case, whereas in another case, when the count was 1.6 million per ml., the reduction time was 1.75 hours. The most typical flavor was produced at 68° F., but higher temperatures of 80° F. and 100° F. developed the flavor more rapidly. Total bacterial counts of samples incubated at 68° F., 85° F. and 100° F. were approximately 5.7 million, 10.4 million and 9.8 million per ml., respectively, when the malt-like flavor was detected.

GENERAL EXPERIMENTAL PROCEDURE

The milk used in this study was secured from the raw milk supply of the Michigan State College creamery and represents a wide variety of sources.

The milk was warmed to the specified temperature (either 32–35° C. or 22–24° C.) and observations made at frequent intervals for flavor changes.

The organoleptic examination of the milk was accomplished by at least two judges working independently. The milk was judged on the basis of the appearance of any acid-associated flavors. Thus, the classification included such flavors as "malty" as well as the typical acid or sour-milk flavors. Agreement between the judges was necessary before any sample was deemed to have an acid-associated flavor.

In addition to taste-odor determinations, titrable acidity and lactic acid measurements, the direct microscopic clump count and the methylene blue reduction test were made.

Lactic acid determinations were conducted essentially by the Hillig technique (6, 7) with certain modifications (2). When it was impractical to analyze for lactic acid immediately, the sample was preserved with mercuric chloride (added at a concentration of 0.3 per cent) and stored at 4° C. The samples were held usually no longer than 48 hours. Results of the lactic acid determinations are referred to as "lactic acid values" unless correction is made for the residual or blank value equivalent to 1–3 mg. lactic acid/100 gm., characteristic of strictly fresh milk (2).

Titrable acidity was ascertained by titrating an 18-gm. sample with 0.05 N NaOH solution to the phenolphthalein end-point, a method considerably more sensitive than the ordinary procedure which involves a 9 gm. sample and 0.1 N NaOH. In addition, titrations were conducted against a white background with good lighting afforded by a desk lamp with a daylight bulb, which was adjusted directly over the samples.

The methylene blue reduction test and the microscopic bacterial clump count were conducted in accordance with Standard Methods (12). All samples were placed in test tubes and held in ice water for 20–30 minutes just prior to adding the dye or before shaking for making the smear for the direct microscopic count. The microscopic clump count was made independently by both authors.

EXPERIMENTAL RESULTS

(*a*) *Effect of Storage at 32–35° C.*

Flavor and lactic acid. In the first trials which were conducted, raw milk from various sources was held at 32–35° C. Samples of the milk were taken at the start of the incubation period and at intervals thereafter until the milk had developed pronounced acid-like flavors. Results showing relationships between the flavor and lactic acid content of the milk are presented in tables 1 and 2.

The total lactic acid content of the milk samples is shown in table 1. These results reveal the relatively low content of lactic acid required before the acid-like flavors appeared. In general, under these conditions, the criti-

TABLE 1

*Correlation of acid-associated flavors with the lactic acid values when the milk was incubated at 32–35° C.**

Flavor of samples	Number of samples having lactic acid values of					
	0–1.9	2–2.9	3–4.9	5–9.9	10–14.9	15 or more
Normal	6	16	9	0	1	0
Acid	1	2	8	15	5	7

* Lactic acid expressed in milligrams per 100 gms. milk.

cal lactic acid content was approximately 5 mg./100 gm. since at values above this concentration all of the samples (with one exception) were classed as having acid-like flavors. However, these data show that samples having such flavors may have lactic acid values below that which is normal for strictly fresh milk. For example, three samples which contained less than 3 mg./100 gm. milk of lactic acid were criticized for having acid-like flavors. In another series conducted under similar conditions, four out of twelve samples were found to have acid flavors at these low lactic acid concentrations.

As a rule, there was a fairly good relationship between the intensity of the developed flavor and the lactic acid content. At lactic acid values above 10 mg./100 gm., the judges usually classed the flavor as "pronounced" or "strong," whereas at lower values, "slight" and "distinct" were the usual designations.

In table 2 is shown the relationship between lactic acid production and acid-type flavors. Lactic acid production represents the difference between the original and final lactic acid content of the milk. The results of these 44 samples reveal lactic acid to have been produced in every case by the time the sample was classed as having an acid-associated flavor, but that the amount of acid involved was slight. All but one of the samples which had developed 5 mg. or more of lactic acid possessed acid-like flavors, and 36, or approximately 82 per cent, of all samples which showed lactic acid increases, were classed as acid. Of the 30 samples which developed more than 2 mg.

TABLE 2

*Correlation of acid-associated flavors with production of lactic acid when the milk was incubated at 32–35° C.**

Flavor of samples	Number of samples having lactic acid increases of					
	0–0.9	1–1.9	2–2.9	3–4.9	5–9.9	10 or more
Normal	5	1	0	1	1	0
Acid	2	6	6	2	14	6

* Lactic acid expressed in milligrams per 100 gm. milk.

of lactic acid per 100 gms., 28 were designated as having acid-associated flavors.

The question may be raised whether these samples had already undergone some lactic acid formation before the trials were started. This would appear to be a logical question, since the milk samples were selected at random from milk as it was delivered from various farms to the plant. However, that appreciable lactic acid production had previously occurred is highly unlikely, because the initial lactic acid values of the samples were within the normal range obtained for fresh milk by this method with an average of 2.5 mg./100 gm.; also, the bacterial counts were too low to indicate bacterial fermentation.

It was observed that the "malty" or "bacterial" type of flavor was common. This may be expected in view of the findings of Hammer and Cordes (4) that warm temperatures favor the production of maltiness by strepto-

TABLE 3

Correlation of bacterial clump count with lactic acid production in milk held at 32–35° C.

Lactic acid increase	Number of samples having microscopic clump counts* of					
	Less than 2	2–2.9	3–4.9	5–9.9	10–19.9	More than 20
mg./100 gm.						
0– 2.0	7	4	3	1	1	...
2– 3.9	1	4	...	1	1	...
4– 5.9	...	1	1	1	3	1
6– 7.9	...	1	...	1	...	2
8– 9.9	...	...	1	...	...	...
10–14.9	...	...	1	1	...	...
15–19.9	...	...	...	1	...	...

* Bacterial count in millions.

cocci. However, the predominant organism in the majority of the "malty" samples appeared to be a bacillus.

Bacteria, lactic acid and flavor. Correlation of the bacterial clump count with the lactic acid production is presented in table 3 and reveals only a general relationship. The relationship would be affected by differences in lactic acid fermenting capacity of the organisms involved and also by the fact that these organisms vary greatly in their clumping ability, thus directly influencing the microscopic count. These results do indicate, however, that appreciable bacterial multiplication had occurred by the time two or more milligrams of lactic acid had been formed, since the milk samples usually had initial bacterial counts well under 200,000 per ml. and often had counts of less than 50,000 per ml.

The relationship between the methylene blue reduction time and lactic acid formation is shown by the data in table 4.

These results reveal that when four milligrams or more of lactic acid had been formed, the methylene blue reduction time was always less than one

TABLE 4

Correlation of lactic acid production with methylene blue reduction time when the milk was held at 32–35° C.

Lactic acid increase	Number of samples having methylene blue reduction time of			
	More than 3.5 hrs.	1–3 hrs.	30 min.–1 hr.	Less than 30 min.
mg./100 gm.				
Less than 2	2	8	4	...
2–3.9	...	3	1	2
4–9.9	...	...	8	5
10 or more	...	...	...	6

hour, and usually was less than 30 minutes. Although the relationship between bacterial count and methylene blue reduction time is not shown, it was observed that all samples having more than two million bacteria reduced the dye within three hours, and usually in less than one hour. The methylene results, however, represent somewhat abnormal conditions since the milk was held at 32–35° C., and had been placed in ice water only 15–30 minutes prior to starting the test. Thus, the organisms were in a highly active state, and would be expected to reduce the dye rapidly.

Another comparison made in these trials involved the microscopic clump count and acid-associated flavors. These results are shown in table 5.

These data reveal that the clump count of milk is relatively low when acid-associated flavors are first perceptible. The majority of samples having counts above two million were graded as acid-flavored; in fact, six samples which possessed acid-like flavors had bacterial counts of less than two million. All samples possessing a clump count of more than five million had acid-associated flavors.

(*b*) *Effect of Storage at 22–24° C.*

Since the possibility exists that storage at 32–35° C. may produce changes which are more or less specific for these temperatures, it appeared desirable to conduct somewhat similar trials in which the milk was held at lower temperatures.

TABLE 5

Correlation of bacterial clump count with production of acid-associated flavors when milk was held at 32–35° C.

Flavor of samples	Number of samples having microscopic clump counts* of						
	Less than 0.5	0.5–.9	1–1.9	2–2.9	3–4.9	5–9.9	10 or more
Normal	18	7	2	0	1	0	0
Acid	0	0	6	13	5	8	11

* Bacterial counts expressed in millions.

In these trials, samples of milk were taken at random as received at the College Creamery, examined for flavor, and analyzed for titrable acidity. They were then stored overnight at 4° C. Early the following morning, the samples were warmed to 22–24° C. and examined at intervals for the pres-

TABLE 6

Relationship between the total lactic acid content of milk and the production of malty and high-acid flavors, the bacterial clump count and the methylene blue reduction test when the milk was held at 22–24° C.

Sample No.	Acid-associated flavor*	Lactic acid content	Microscopic clump count		Methylene blue reduction time
			Number	Predominant type†	
		mg./100 gm.	*millions*		*hours*
1	Malty +	2.5	1.4	Rods	2.8
2	" ++	1.6	3.3	Rods, cocci	1.5
3	" ++	4.5	6.4	Rods	2.3
4	" ++	2.2	2.8	Rods, cocci	3.5
5	" +	2.3	3.0	Rods, cocci	2.5
6	" +	2.5	5.2	Rods	1.7
7	" +	4.6	6.9	Rods, cocci	0.9
8	" +	4.6	2.9	Rods, cocci	2.5
9	" ++	2.9	6.1	Rods, cocci	1.5
10	" ++	4.6	6.7	Rods	0.9
11	" ++	5.7	6.6	Rods, cocci	0.9
12	" ++	3.8	4.5	Strep.	2.5
13	Malty, high-acid +	2.3	7.5	Rods, cocci	0.7
14	" " +	2.9	2.4	Rods, cocci	3.3
16	" " +	4.8	1.5	Rods, cocci	2.5
17	" " ++	.....	12.0	Rods, cocci	1.0
18	" " +	.....	3.8	Rods, cocci	1.5
19	" " +	2.5	1.3	Rods, cocci	2.0
20	" " +	.....	2.3	Strep., staph.	1.9
21	" " +	8.2	7.3	Rods, cocci	0.9
22	" " ++	3.1	14.3	Rods, cocci	0.9
23	" " +	4.1	4.5	Rods, cocci	1.5
24	" " ++	4.6	19.1	Rods, cocci	0.7
25	" " +	3.1	6.6	Rods	0.9
26	High-acid ++	10.9	20.0	Strep.	0.3
27	" +	3.6	10.0	Strep., staph.	1.2
28	" +	10.2	18.8	Strep., rods	2.3
29	" +++	25.0	61.4	Strep.	0.3
30	" +	5.6	21.0	Strep.	0.7
31	" +++	16.9	62.0	Strep.	0.3
32	" +	4.7	11.7	Strep., rods	0.9
33	" +++	25.9	160.0	Strep.	0.5
34	" +++	24.4	131.0	Strep.	0.3
35	" ++	18.2	68.5	Strep., rods	1.0

* Intensity of flavor indicated by number of plus signs: + slight; ++ distinct; +++ pronounced.

† Unless otherwise indicated, "cocci" refers to various types of coccus-formed bacteria other than streptococci, *i.e.*, micrococci, diplococci, and in certain cases, staphylococci.

ence of acid-associated flavors. When a sample exhibited such a flavor it was promptly subjected to the various quality tests.

Lactic acid, flavor, bacterial tests. The examination of these samples for developed flavors revealed that although the malty flavor appeared fre-

quently, as in the case of the higher storage temperature, there were more samples in which a typical acid flavor developed. This high-acid flavor appeared to be more difficult to detect than those flavors of the malty type and, therefore, was associated with higher titrable acidity increases and with much greater bacterial counts. In these trials, the judges attempted to differentiate between the flavors by the terms "malty" and "high-acid." The results showing the relationship between the total lactic acid content of milk and the flavors, bacterial count, and methylene blue test, are presented in table 6.

In this table, the samples were grouped in accordance with the flavor defect indicated by the judges, *i.e.*, malty, malty-high-acid and high-acid. However, recognition should be given to the fact that it is not always possible to differentiate between these various acid-associated flavors. The data in table 6 indicate that for the same intensity of off-flavor, the high-acid samples had undergone more lactic acid formation and possessed definitely higher bacterial counts than those samples having the malty-type flavor. There are, however, some exceptions.

There is no appreciable difference between the lactic acid values and bacterial counts of the two groups classed as "malty" and "malty-high-acid." Of the 12 samples possessing slight or distinct malty flavors, the lactic acid content ranged from 1.6 mg. to 5.7 mg./100 gm. and the bacterial counts ranged from 1.4 million to 6.9 million/ml. In the "malty-high-acid" group, the lactic acid content ranged from 2.3 mg. to 8.2 mg./100 gm. and the bacterial count ranged from 1.3 million to 10.1 million/ml. The average values for all of these samples having at least some semblance of a malty flavor were as follows: Lactic acid content, 3.7 mg./100 gm.; bacterial count, 5.7 million/ml.; methylene blue reduction time, 1.7 hours.

Although the milk was examined at frequent intervals, several of the "high-acid" samples had developed a pronounced flavor by the time of detection, probably because of the extremely high bacterial counts and the accelerated bacterial activity. The flavor production likely occurred rapidly between observation periods. It is interesting to note that in those samples having pronounced high-acid flavors, the bacterial count ranged from 61.4 million to 160 million/ml. and the lactic acid content ranged from 16.95 mg. to 25.90 mg./100 gm. Average values for the 10 samples characterized as "high acid" were as follows: Lactic acid content, 14.5 mg./100 gm.; bacterial count, 56.4 million/ml.; methylene blue reduction time, 0.8 hour.

Another interesting deduction which may be drawn from the results in table 6, pertains to the type of organism which predominates in production of the flavors. In those samples having malty-like flavors, rod-shaped organisms were involved in all but one case, and they were usually accompanied by smaller numbers of cocci of one or more types. It should be emphasized, however, that the organism which predominates under conditions such as

exist in this experiment may be influenced by the temperature and time of incubation.

The correlation between bacterial count and lactic acid production is seen to be only fairly general. For example, in one case, a sample with a bacterial count of 131 million/ml. had a lactic acid value of 24.4 mg./100 gm. in contrast to another sample with a count of 61 million/ml. and a lactic acid value of 25 mg./100 gm. In both of these samples, however, the bacterial count and the lactic acid content were comparatively high. If these were compared with a sample having a bacterial count of 10 million, a definite difference in the lactic acid values would be expected.

The results with the methylene blue test indicate that, under the conditions of this experiment, milk having a high lactic acid content will usually exhibit short reduction periods. For example, milk with lactic acid value of 15 mg./100 gm. or more, reduced the dye in one hour or less. However, milk having far less lactic acid may also reduce the dye quickly. It does appear

TABLE 7

*Increases in lactic acid as measured by titrable acidity and by lactic acid methods**

Range in lactic acid content	No. of samples	Average lactic acid increase		Range of individual sample variation (b) from (a)
		By lactic acid method (a)	By titrable acidity (b)	
mg./100 gm.		*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
4.9 or less	36	1.6	2.4	0.3–10.3
5.0– 9.9	18	6.8	8.5	0.2–13.3
10.0–14.9	3	14.3	13.5	0.6– 9.7
15.0–19.9	4	17.9	22.2	0.2–14.8
20 or more	5	22.8	26.7	1.4– 6.5

* Titrable acidity determination was conducted on an 18-gm. sample with 0.05 N NaOH.

likely, however, that milk which reduces the dye within 30 minutes will contain appreciable quantities of lactic acid.

Titrable acidity and lactic acid. At the beginning of this investigation, an effort was made to use both pH measurements and the titrable acidity test to measure acid production in the milk. However, the pH determinations were soon discontinued when they were found to be no more sensitive than the titration test in detecting slight acidity changes.

In the case of acidity measurements, it is fully realized that these determinations are of no value insofar as establishing grading standards therefrom is concerned, since normal variations in titrable acidity far exceed the changes required to produce appreciable quality deterioration in milk. However, the principal purpose of such determinations was to permit a direct comparison between acidity increases as measured by titration and as measured by the more sensitive lactic acid technique.

As previously indicated, the titrable acidity determination involved an 18-gm. sample and 0.05 N NaOH. The results of 66 trials in which compari-

sons were made between increases in lactic acid and increases in the titrable acidity (also expressed as lactic acid) are summarized in table 7. In this table, data are arranged to indicate the variation of the titrable acidity from the true lactic acid content at different levels of lactic acid increases.

These data reveal a fairly close relationship to exist between the two methods when the average results are considered, with a maximum difference of approximately 4 mg./100 gm. However, the data also show that in the case of individual samples, the titrable acidity results may vary rather widely from the true lactic acid content, the maximum variation amounting to 14.8 mg. lactic acid per 100 gm. milk.

Although the results of the titration and lactic acid methods on individual milks are not shown, the former was found to give lower results in 29 of the 66 samples, or in 43.9 per cent of the total determinations. Furthermore, it was observed that the extreme variations between the two methods occurred in those cases in which the titration values were higher. In samples in which the titration results were lower than obtained by the lactic acid procedure, the maximum variation was approximately 5 mg. lactic acid/100 gm. However, in the 37 samples in which the titration indicated a higher lactic acid content than shown by the lactic acid method, the maximum difference between the methods was 14.8 mg./100 gm., and seven samples varied by more than 8 mg./100 gm.

Two factors are likely involved in creating the variations between these two methods on individual samples: (a) the lack of accuracy on the part of the titration procedure, and (b) the possibility that the development of rancidity in certain samples before the acid flavor appeared may have influenced the titration result. Even by using the titration technique involving larger samples, weaker alkali solution, and excellent lighting, the titration method is still subject to some end-point errors and variations. That the titration results agree to the extent indicated in table 7 is surprising.

From the rancidity standpoint, certain samples did possess rancid flavors early in the storage period, and the development of rancidity may be expected to increase the titrable acidity without affecting results by the lactic acid determination. This factor is likely responsible for the major differences between the two methods.

DISCUSSION

When consideration is given to the grading of the raw milk supply on the basis of the lactic acid content, the question which arises is, "When is milk sour?" and the answer to the question will likely be an important consideration if and when quality standards involving lactic acid are established. However, at the present time a direct simple answer to this question is difficult, if not impossible, because any such answer must be qualified in one manner or another as dictated by the various factors involved.

One factor to be considered in this connection concerns the methods used for determining the degree of "sourness" or "sweetness" of milk. Under present conditions, milk that is deemed to be sour by one method may not be classed as sour by another. Therefore, if the extent of sourness is to be used as the basis of a grading program in the concentrated milk field, then the various practical quality methods now in use should be reinvestigated with the view of establishing standards for each of them on this basis.

Organoleptic detection of sourness in milk has been the subject of much discussion, and its reliability is questioned because of (a) differences in the acuteness of the individual's senses of taste and smell; (b) the presence of other flavors which may interfere with sour flavor detection; (c) peculiarities of specific flavors produced by different microorganisms, these flavors being detectable at varying levels of acid development. Notwithstanding, the results obtained in this study indicate that, when properly applied, the organoleptic method is highly sensitive and detected acid-type flavors which occurred as a rule at much lower lactic acid levels than have been reported previously. Generally, when acid-associated flavors were perceptible, lactic acid increases were below 10 mg./100 gm. and often were not over 5 mg./100 gm. In fact, lactic acid increases of 2 mg./100 gm. were not uncommon in milk having acid-associated flavors.

On the basis of available information, it appears that a grading standard which permits an increase in the milk of 0.03 per cent acidity (30 mg./100 gm. of lactic acid) is indeed generous if the grading is to be conducted by well-trained milk graders. This amount of formed acid is considerably above the critical concentration for flavor production. It is appreciated, however, that a certain tolerance above the critical lactic acid content found in this study is desirable, since it is not always feasible to have the grading conducted by highly skilled milk judges. In addition, more information is desirable on the flavor-acid relationship as influenced by different microorganisms before establishing stringent lactic acid standards.

As has been pointed out by previous workers, the actual taste and odor detectable at the first signs of souring are probably due to compounds other than lactic acid. However, the results herein reported indicate that lactic acid has been formed in practically all, if not all, samples of milk which possess an acid-associated flavor, although the amount formed is often slight.

The type of flavor produced in milk during souring is a factor to be considered in arriving at conclusions regarding organoleptic grading of such milk. The authors have often observed that flavors of the malty type are detected at lower lactic acid content and lower bacterial count than those flavors which are more strictly of the high-acid type, doubtless due to the larger volume of aroma associated with the malt-flavor production. This field of study needs further exploration before final conclusions may be drawn.

One point of contention in milk grading relates to the terminology used in designating the sour-milk flavors. The term "high-acid" is objected to by many because the milk is within the normal range of titrable acidity. Furthermore, the term "high-acid" indicates an acid-like flavor, whereas there is no semblance of an acid flavor in the earlier stages of souring. It would appear logical, at least for a quality program, to classify all of those flavors which occur when milk undergoes souring as one general type, and avoid the confusion which may result by differentiation. The principal point involved is that these flavors are all produced by bacterial activity coincident with the formation of lactic acid and the appearance of any of these flavors indicates milk of inferior quality. The term "acid-associated" would appear to be suitable for all of those flavors which result from or occur simultaneously with lactic acid formation.

The relationship found in this investigation between bacterial count and flavor appears to be somewhat different than has been indicated previously. The results do reveal that acid-associated flavors are commonly present in milk having bacterial clump counts of 2–5 million per ml. These comparatively low values, considerably under the plate counts reported by Hammer and Hix (5), may be the result of (a) detecting the flavor changes at lower acid increases, (b) allowing the milk to develop the flavor normally with a wide variety of organisms being present and without resorting to artificial inoculation, and (c) differences in the types of organisms involved. The results herein presented indicate that the production of about 0.02 per cent titrable acidity (20 mg. lactic acid/100 gms. milk) by a predominance of streptococci, gave data similar to those of Hammer and Hix (5).

Variation in bacterial counts may be normally expected and, in this study, milk samples exhibited widely different microscopic clump counts at the first detectable flavor change. These variations, in general, are governed by the type of organism predominating in the respective samples and also by the temperature of incubation. Certain organisms possess considerable clumping properties and remain in clumps during the preparation of the sample for staining. Other organisms, particularly the streptococci found in these samples showing a typical "high-acid" flavor, were more widely distributed over the microscopic field. Thus, in two samples, the individual cell count may be similar, but due to the clumping properties of the predominant organism in one sample, the clump counts may be markedly different.

The titrable acidity test as it is now used will probably never be of any great value as an accurate control test, since the amount of the titer which is contributed by lactic acid is not easily ascertained. In fact, the common titration procedure which involves the use of a 9-gm. sample and 0.1 N NaOH solution would appear to be of little value even as an adjunct to organoleptic examination unless the sample in question had undergone

acidity changes far in excess of those required for production of acid-associated flavors. For example, the results of these experiments indicate that the milk usually was of inferior quality as determined by any of the quality tests when the lactic acid increase amounted to 0.010 per cent or less. Since the titrable acidity of mixed herd milk may normally vary by several times this quantity, it may readily be appreciated that the establishment of grading standards based on titrable acidity measurements is unfeasible.

In connection with acidity values, the results herein presented reveal a general relationship between acid increases as measured by a sensitive titration method and by the lactic acid method. However, under normal conditions, even a sensitive titration method should serve only as an indicator of acidity changes rather than as a precise instrument for measuring lactic acid changes, since titrable acidity increases on individual milk samples may vary appreciably from the true lactic acid content.

SUMMARY AND CONCLUSIONS

Results are presented of the relationship of the formation of lactic acid in milk with (a) the production of acid-associated flavors, (b) the microscopic clump count, (c) the methylene blue reduction, and (d) titrable acidity.

Acid-associated flavors were detected at low levels of lactic acid formation. Many samples of milk exhibited such flavor defects at lactic acid increases of 2–5 mg./100 gm. or less and practically all samples possessed acid-associated flavors of lactic acid values of 10 mg./100 gm. However, the type of flavor, the temperature of incubation, and the type of organisms may somewhat influence these values.

Bacterial clump counts, in general, range from about 1.5 to 10 millions per ml. in those samples showing slight acid-associated flavors. The same factors which influence the acid flavor-lactic acid relationship are also of importance in this connection.

The methylene blue reduction time was markedly shortened in all samples of milk showing appreciable increases in lactic acid.

A general relationship was noted in the lactic acid increases obtained by the colorimetric lactic acid method and by titration. However, the titration results may vary appreciably from the true lactic acid changes in individual milks.

A discussion is given of the limitations and application of certain of these quality tests in a lactic acid grading program.

REFERENCES

(1) Cooledge, L. H., and Wyant, R. W. The Keeping Quality of Milk as Judged by the Colorimetric Hydrogen Ion Concentration. Jour. Dairy Sci., 3: 156–166. 1920.

(2) GOULD, I. A. Lactic Acid in Dairy Products. I. Application of the Hillig Method. JOUR. DAIRY SCI., 27: 743–752. 1944.

(3) HAMMER, B. W. Dairy Bacteriology, 2nd Ed., 482 pp. illus. John Wiley and Sons, Publishers. 1938. p. 31.

(4) HAMMER, B. W., AND CORDES, W. A. Burnt or Caramel Flavor in Dairy Products. Iowa Agr. Expt. Sta. Res. Bul. 68. 1921.

(5) HAMMER, B. W., AND HIX, B. H. Studies on the Numbers of Bacteria in Milk Showing Various Changes. Iowa Agr. Expt. Sta. Res. Bul. 29. 1916.

(6) HILLIG, F. The Colorimetric Determination of Lactic Acid in Milk and Milk Products. Jour. Assoc. Off. Agr. Chem., 20: 130–140. 1937.

(7) HILLIG, F. Colorimetric Determination of Lactic Acid. Jour. Assoc. Off. Agr. Chem., 26: 199. 1943.

(8) RICHMOND, H. D., AND HARRISON, J. B. P. Notes on Sour Milk. Analyst, 25: 116–124.

(9) SCHULTZ, E. W., MARX, A., AND BEAVER, H. J. The Relationship between Hydrogen-Ion Concentration and the Bacterial Content of Commercial Milk. JOUR. DAIRY SCI., 4: 1–6. 1921.

(10) SOMMER, H. H. Market Milk and Related Products. Published by the Author, Madison, Wis. pp. 150–151.

(11) SOMMER, H. H., AND HART, E. B. Grading Milk by the Acid Test: Influence of Acids in the Ration on the Acidity of Milk. JOUR. DAIRY SCI., 4: 7–11. 1921.

(12) STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS. 8th Ed. Published by the American Public Health Assoc., N. Y. 1941.

(13) STEUART, D. W. The Development of Acidity in Milk. JOUR. DAIRY SCI., 3: 52–59. 1920.

(14) STOKES, A. W. The Influence of Milk Preservatives. Analyst, 16: 122–126. 1891.

(15) TRACY, P. H., AND RAMSEY, R. J. Malt Flavor in Raw Milk Produced by Micrococcus. JOUR. DAIRY SCI., 14: 457–462. 1931.

(16) VAN SLYKE, L. D., AND BAKER, J. C. Free Lactic Acid in Sour Milk. Jour. Biol. Chem., 35: 147–178. 1918.

A TECHNIQUE FOR THE SIMULTANEOUS MEASUREMENT OF SEMEN QUALITY AND TESTIS HISTOLOGY IN VITAMIN A STUDIES OF THE DAIRY BULL*

R. E. ERB, F. N. ANDREWS, J. F. BULLARD AND J. H. HILTON

Purdue University Agricultural Experiment Station, Lafayette, Ind.

Vitamin A deficiency is the most common of the known vitamin deficiencies which occur in cattle. The clinical symptoms, gross and microscopic pathology and therapy of vitamin A deficiency have been reported by many investigators and are not reviewed in this report.

It has long been known that vitamin A deficiency results in the cessation of spermatogenesis and atrophy of the testis in laboratory animals (8, 9) and in cattle recent studies (3, 4, 5, 6, 7) have shown vitamin A deficiency to have a similar effect on the testis of the bull.

When small numbers of animals are available it is obviously necessary to obtain as much information from each individual as possible. The standard procedures which have been developed for the measurement of semen quality are of unquestioned value but it is especially desirable to be able to determine the effects of vitamin A deficiency and therapy upon the spermatogenic tissue of the testis itself. This has formerly been possible by autopsy or by the surgical removal of one testis. Each of these methods has the common disadvantage of the limited number of times it can be applied to a particular animal. In recent years the development of biopsy techniques whereby small pieces of tissue can be removed at various intervals have made it possible to gather large amounts of data from single animals. Andrews and McKenzie (1) performed as many as 28 biopsies upon the genitalia of a single mare within a three-month period.

The purpose of this report is to describe the procedures used in the conduct of vitamin A studies in the dairy bull. The results obtained in this study should be regarded as preliminary in nature but it is believed that the method will be of value to other investigators in this field.

EXPERIMENTAL PROCEDURES AND RESULTS

Semen was collected at weekly intervals using an artificial vagina. Two successive samples were obtained except in some cases when the bull refused to ejaculate twice. Semen examinations included volume of ejaculate, concentration and total numbers of spermatozoa, initial sperm motility, the maintenance of motility when the semen was stored at 40° F. and abnormal sperm forms. The details of the techniques have been published by Erb *et al.* (2). Testis tissue was removed by biopsy from the bull twice during

Received for publication February 26, 1944.

* Journal paper No. 153, Purdue University Agricultural Experiment Station.

the experiment. The testicular biopsies were performed by making a small incision in the lateral scrotal wall, exposing the tunica albuginea and, with a sharp scalpel, excising a testicular section about 5 mm. square. The testis usually bled freely but no special attempt to control bleeding was made and clotting occurred spontaneously. The tunica albuginea can, but need not, be sutured and one or two sutures can be placed in the scrotal wall. Recovery from the operation was uneventful in each case. The testicular tissue was immediately fixed in Bouin's fluid, dehydrated in an ethyl-butyl alcohol series, embedded in paraffin, sectioned and stained in Mallory's triple connective tissue stain.

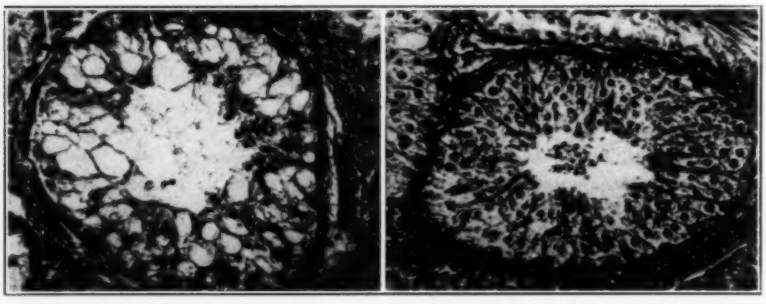


FIG. 1. The germinal epithelium has nearly completely disappeared and only a few spermatogonia are present. × 220.

FIG. 2. The germinal epithelium has been greatly repaired and spermatozoa are present.

The bull used in this study was a crossbred Holstein-Guernsey born May 4, 1941. During the six-month period preceding the experiment, he was fed the standard Purdue growing ration supplemented with cod liver oil and was, to the best of our knowledge, a normal healthy animal. On June 8, 1942, the bull was placed on a vitamin-A-low ration composed of beet pulp as the only roughage and a grain mixture of white corn, oats, linseed oil meal, bonemeal and salt. On October 8, 1942, symptoms of advanced vitamin A deficiency were observed. The bull became blind, lost the coordination of movement and showed such gastro-intestinal disturbances as diarrhea and bloat. A testicular biopsy obtained from the right gonad on October 12 showed severe degeneration of the seminiferous tubules and the nearly complete disappearance of sperm from the lumena of the tubules (fig. 1). As summarized in table 1 semen quality decreased steadily until December 7, 1942.

Vitamin A therapy was begun on October 12, 1942, and the following treatment was given: October 12, 1942, to December 8, 1942, 60,000 I.U. of vitamin A daily; December 8, 1942, to January 5, 1943, 100,000 I.U. daily; January 5, 1943, to March 20, 1943, 150,000 I.U. daily.

A second section was removed from the right testis on February 5, 1943. Microscopic examination revealed that extensive but not complete repair of the seminiferous tubules had occurred (fig. 2). As shown in table 1 semen quality began a gradual improvement during the latter part of December. It can be observed that semen quality continued to decline after the initiation of vitamin A therapy on October 12, and that no spermatozoa were obtained in a series of 4 ejaculates collected during the interval ending

TABLE 1

Semen production of bull L. H. on a vitamin A low diet and after vitamin A therapy

Interval ending	No. semen samples	Ave. vol.	Ave. sperm per cu. mm.	Ave. total sperm	Ave. initial sperm motility	Ave. sperm survival	Ave. abn. sperm per 1000
		cc.	*thousand*	*million*		*days*	
1942							
6–23	4	1.2	98	137	1.5	2.0	118
7–7	4	1.5	370	631	4.3	6.3	97
7–21	4	0.9	728	914	4.3	6.5	114
8–4	4	1.3	973	1244	4.8	14.0	101
8–18	4	1.1	793	961	4.0	7.0	97
9–1	4	0.7	913	585	3.8	6.0	55
9–14	4	0.4	435	230	3.0	4.0	88
9–29	4	1.0	730	834	2.0	4.3	132
10–12	...	.....	.....	.....	...	...	.....
10–26	4	1.0	220	229	1.3	1.0	408
11–9	4	1.4	20	27	0.5	0.5	.....
11–24	4	1.3	3	2	0	0	.....
12–7	4	1.5	0	0	0	0	...
12–21	4	1.2	40	79	1.0	1.0	261
1943							
1–5	4	1.3	83	124	0.8	0.8	189
1–18	4	1.1	288	386	2.3	2.3	243
2–1	6	1.5	762	1064	2.7	4.7	230
2–16	4	1.4	890	1380	1.0	2.8	267
3–1	4	1.9	245	745	1.0	2.8	350
3–16	4	1.9	98	245	2.0	1.5	159
3–22	4	0.5	115	108	2.0	1.8	142
4–7	4	2.4	683	1072	2.3	3.5	284

December 7. This can be theoretically explained as representing the gradual depletion of sperm stored in the vas deferens and epididymis. It can likewise be theorized that the lag in improvement of semen quality after the beginning of vitamin A treatment may have represented the time necessary for the restoration of the damaged germinal epithelium and the subsequent filling of the epididymis with newly formed spermatozoa.

It seems, therefore, that the effects of vitamin A deficiency and therapy upon spermatogenesis in the dairy bull can be effectively measured by the simultaneous study of the semen and the microscopic anatomy of the testis.

REFERENCES

(1) ANDREWS, F. N., AND MCKENZIE, F. F. Estrus, Ovulation and Related Phenomena in the Mare. Mo. Agr. Expt. Sta. Res. Bul. 329. 1–117. 1941.

(2) ERB, R. E., ANDREWS, F. N., AND HILTON, J. H. Seasonal Variation in Semen Quality of the Dairy Bull. JOUR. DAIRY SCI., 25: 433–440. 1942.

(3) GUILBERT, H. R., AND HART, G. H. Minimum Vitamin A Requirements with Particular Reference to Cattle. Jour. Nutr., 10: 409–427. 1935.

(4) HART, G. H. Vitamin A Deficiency and Requirements of Farm Mammals. Nutr. Abs. and Rev., 10: 261–272. 1940.

(5) MADSEN, L. L., HALL, S. R., AND CONVERSE, H. T. Cystic Pituitary in Young Cattle with Vitamin A Deficiency. Jour. Nutr., 24: 15–24. 1942.

(6) SUTTON, T. S., KRAUSS, W. E., AND HANSARD, S. L. The Effects of Vitamin A Deficiency on the Young Male Bovine. JOUR. DAIRY SCI., 23: 574. 1940.

(7) THORP, W. T. S., KEENER, H. A., BECHDEL, S. I., AND GUERRANT, N. B. Observations on the Pathology of Dairy Calves on Low Vitamin A Diets. Amer. Jour. Vet. Res., 3: 27–31. 1942.

(8) WOLBACH, S. B., AND BESSEY, O. A. Tissue Changes in Vitamin Deficiencies. Physiol. Revs., 22: 233–289. 1942.

(9) YAMASAKI, Y. Experimentelle Untersuchung über den Einfluss des Vitaminoder Zellsalzmanzels auf die Entwicklung von Spermatozoen and Eiern. Arch. f. Path. Anat. u. Physiol., 245: 513. 1923.

SEASONAL AND GEOGRAPHICAL VARIATIONS IN THE CITRIC ACID CONTENT OF DEFATTED MILK SOLIDS

BURDET HEINEMANN

Producers Creamery Company, Springfield, Missouri

The literature on the citric acid content of milk shows a wide range in values. Allen (1) attributes the variations to the unreliable character of the methods of analysis and the analysis of samples which have undergone citric acid fermentation. He points out that the range reported is far out of proportion to the differences exhibited by the other constituents of milk.

Since the determination of the milk solids content of bread by means of its citric acid content has been suggested by Hartman and Hillig (5) and since the use of milk solids has been extended to a wide variety of other products, a study was made to determine the extent of seasonal and geographical variations in the citric acid content of defatted milk solids.

REVIEW OF LITERATURE

The number of studies dealing with seasonal and geographical variations in milk is limited. Sherwood and Hammer (9) made 335 determinations on whole milk from individual cows and concluded: "There is no significant seasonal variation in the citric acid content of the milk." Their values ranged from 0.07 to 0.33 and averaged 0.18 per cent. Arup (2) analyzed 25 samples of fresh retail milk taken over a year's time. These values ranged from 0.150 to 0.206 per cent and did not indicate significant seasonal variation. He also determined the citric acid content of 11 samples of defatted milk solids of English, Irish, and American origin. There was no significant geographical variation, his results ranging from 0.151 to 0.184 per cent on the reconstituted basis.

A composite sample of 5 cows' milk on vitamin-C-free feed had a citric acid content of 0.08 per cent compared to 0.13 per cent citric acid in the milk from the same cows on pasture, according to Hess *et al.* (7). Supplee and Bellis (10) also found more citric acid in milk from cows on pasture (0.148%) than from cows on winter feed (0.142%).

Hartman and Hillig (4) conducted a collaborative study of the milk supply of 14 large cities located in various parts of this country and found that the average citric acid content of 58 samples was 0.16 per cent. These samples ranged from 0.14 to 0.19 per cent and exhibit some geographical variation, San Francisco showing the lowest and New Orleans the highest per cent citric acid.

Holm, Webb, and Deysher (8) studied the citric acid content of the milk from 4 cows throughout the lactation period. Their results indicated that

Received for publication February 28, 1944.

TABLE 1

Seasonal variation in citric acid content of defatted milk solids, 1943 (moisture-free basis)

Date	Citric acid (anhydrous)	Date	Citric acid (anhydrous)
	per cent		*per cent*
1/4	2.04	7/29	2.00
	2.05		1.98
1/16	2.02	8/2	1.95
	2.02		1.97
1/31	1.95	8/15	1.97
	1.95		1.95
2/11	2.00	8/20	1.95
	2.01		1.94
2/28	1.99	8/25	1.97
	1.98		1.99
3/9	2.00	9/15	2.01
	2.02		2.01
3/17	2.08	9/30	2.00
	2.09		1.99
4/9	2.09	10/20	1.98
	2.09		2.00
4/30	2.05	10/31	1.92
	2.06		1.92
5/12	2.04	11/11	1.89
	2.08		1.88
5/26	2.02	11/26	1.88
	2.04		1.88
6/15	2.06	12/9	1.95
	2.05		1.97
6/23	2.04	12/24	1.96
	2.04		1.97
7/15	2.00	*Average*	1.995

TABLE 2

Geographical variations in citric acid content of defatted milk solids, July, 1943 (moisture-free basis)

State	Citric acid (anhydrous)	State	Citric acid (anhydrous)
	per cent		*per cent*
Wisconsin	1.97	Mississippi	1.98
	1.97		1.99
Pennsylvania	2.05	Utah	1.89
	2.07		1.91
Minnesota	2.07	Michigan	1.84
	2.09		1.83
Missouri	2.01	Idaho	1.86
	2.02		1.90
Maryland	1.95	Oregon	1.98
	1.94		1.96
California	1.93	*Average*	1.961
	1.93		

there was a decrease in citric acid as the period of lactation progressed. Their values ranged from 0.19 to 0.29 per cent.

In a recent review of the literature, Hammer and Babel (3) concluded, "While the citric acid content of milk shows some variation, it appears that herd milk commonly contains from 0.16 to 0.18 per cent."

EXPERIMENTAL

The method used for the determination of citric acid in dry milk solids has been described in a previous publication (6). This method is based on the oxidation of the citrate ion by the perchlorate-cerate ion.

The samples of dry milk solids used in the seasonal variation study were collected from January, 1943, to January, 1944, at this plant. Each sample represents a composite of a large amount of milk. All determinations were made in duplicate.

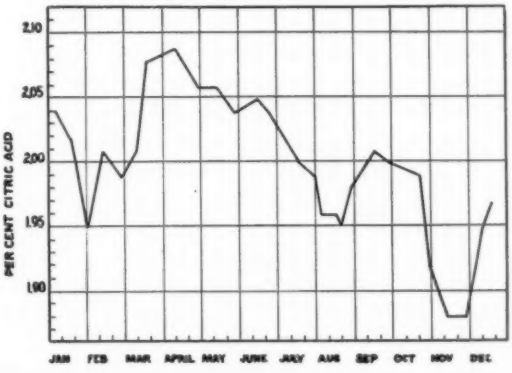


FIG. 1. Seasonal variation in citric acid content of defatted milk solids (moisture-free basis) during 1943.

The samples used to determine geographical variations were secured from the American Dry Milk Institute who received them during the month of July, 1943, from drying plants located in the specified states.

All samples were tested for moisture and lactic acid content and in no case did the latter exceed 0.01 per cent on the reconstituted basis. This indicates that the samples were of sufficiently good quality to preclude the possible loss of citric acid due to fermentation. The moisture was found to vary within the limits of 2.5 and 5.0 per cent, and for this reason, results were calculated to the moisture-free basis.

Figure 1 is a graphical representation of the data presented in table 1, showing the range in seasonal variation. Table 2 shows the geographical range in citric acid content of dry milk solids not fat.

DISCUSSION

The study of the 27 samples taken throughout the year reveals that there is a small but definite seasonal variation in the citric acid content of defatted milk solids, ranging from a maximum 2.09 per cent in March and April to a minimum of 1.88 per cent in November and averaging 1.995 per cent. This does not substantiate the claim of Hess *et al.* and Supplee and Bellis that cows on pasture produce milk containing more citric acid than those on dry feed because a large majority of the cows in this section of the country were still on dry feed in March and April, 1943. These results seem to substantiate the data of Holm *et al.* which indicates that there is a decrease in the citric acid content of the milk as the period of lactation increases inasmuch as a large majority of the cows in this territory freshen in the spring. There are undoubtedly other factors, however, which are also concerned. One is that about 90 per cent of the herds supplying milk to this plant consist of Jersey cows. Another is that in 1943 there was an excessive amount of rainfall during April and May.

The study of the eleven samples taken from various parts of the country shows a range of values from 1.83 to 2.08 per cent and averaging 1.961 per cent. When calculated to a skim milk containing 9.3 per cent total solids, these figures are 0.165 to 0.193 per cent with an average of 0.182 per cent. There does not seem to be a relation between the section of the country in which the milk was produced and its citric acid content. For example, Minnesota has the highest and Michigan the lowest per cent citric acid. Nor is there any agreement between these figures and those of Hartman and Hillig (4). Furthermore, the difference in the range of values, when calculated on the reconstituted basis (0.028%), is not as great as the difference in the range reported by them (0.05%).

When calculated to a reconstituted basis, the range in citric acid in all samples—a total of 38—is 0.170 to 0.201 per cent (average 0.185%). This difference of 0.031 per cent is considerably less than would be expected in the light of previous work in which a difference as great as 0.26 per cent between maximum and minimum values is reported (9). The three most important reasons for this are: first, the use of a method which yielded close checks on duplicate samples; second, the analysis of samples which showed no evidence of fermentation; and third, the selection of samples which represented a composite of the milk from a large number of cows. As pointed out previously (6), the method used may give results slightly higher than the A.O.A.C. method.

It can be assumed, however, that the citric acid content of the defatted milk solids is sufficiently uniform throughout the country to be used as an index for the determination of the milk solids content of bread or other product.

SUMMARY AND CONCLUSIONS

The citric acid content of 27 samples of defatted milk solids taken through the year 1943 varied from 1.88 to 2.09 per cent and averaged 1.995 per cent. The highest value occurred in March and April and the lowest in November, thus indicating a small but definite seasonal variation.

Samples of defatted milk solids taken from 11 different states during July, 1943, varied from 1.83 to 2.08 per cent (average 1.961%), thus indicating that there is a slight variation in the citric acid content of milk from different localities.

The citric acid content of defatted milk solids, however, is sufficiently uniform to be used as an index for the determination of the milk solids content of bread or other products.

REFERENCES

(1) ALLEN, L. A. The Mineral Constituents and Citric Acid Content of Milk. Jour. Dairy Res., 3: 1–51. 1931.

(2) ARUP, PAUL S. Citric Acid Determination in Milk and Milk Products. Analyst, 63: 635–640. 1938.

(3) HAMMER, B. W., AND BABEL, F. J. Bacteriology of Butter Cultures: A Review. JOUR. DAIRY SCI., 26: 83–168. 1943.

(4) HARTMAN, B. G., AND HILLIG, F. Approximation of the Milk Solids of a Milk Product by Means of its Citric Acid Content. Jour. Assoc. Off. Agr. Chem., 16: 427–431. 1933.

(5) HARTMAN, B. G., AND HILLIG, F. Estimation of Milk Solids in Bread. Jour. Assoc. Off. Agr. Chem., 16: 431–435. 1933.

(6) HEINEMANN, BURDET. The Determination of Citric Acid in Milk Products by Cerate Oxidimetry. JOUR. DAIRY SCI., 27: 377–383. 1944.

(7) HESS, A. F., UNGER, L. S., AND SUPPLEE, G. C. Relation of Fodder to the Antiscorbutic Potency and Salt Content of Milk. Jour. Biol. Chem., 45: 229–235. 1920.

(8) HOLM, G. E., WEBB, B. H., AND DEYSHER, E. F. The Heat Coagulation of Milk. JOUR. DAIRY SCI., 15: 331–343. 1932.

(9) SHERWOOD, F. F., AND HAMMER, B. W. Citric Acid Content of Milk. Iowa Agr. Expt. Sta. Res. Bul. 90. 1926.

(10) SUPPLEE, G. C., AND BELLIS, B. Citric Acid Content of Milk and Milk Products. Jour. Biol. Chem., 48: 453–461. 1921.

FURTHER STUDIES ON BLOAT

S. W. MEAD, H. H. COLE, AND W. M. REGAN
Division of Animal Husbandry, University of California, Davis, California

The purpose of this paper is to submit data, supplementing our earlier studies (1, 2, 3, 5), on the cause, production, and prevention of bloat.

EXPERIMENTAL

Because several independent experiments are involved, most of the details on methods will be discussed under the separate sections. A few general statements can be made. Throughout the feeding period, when bloat was anticipated, the cows were constantly observed by at least one and usually two or more persons. We considered it safe to leave animals if bloat had not developed one hour after they were taken off pasture. Animals removed from the pasture and fed grain shortly thereafter would often bloat and occasionally had to be treated. In the series of experiments to be considered, there were 194 cases of bloat. Since the cattle were observed carefully, and were treated at once if seriously bloated, none was lost.

The experimental animals were mostly Holsteins and Jerseys, with two Guernseys and one Ayrshire. All are indicated by herd numbers through which the breed can be identified: those in the 1,000 series are Holsteins; 700 and 800 series, Jerseys; 600, Guernseys; 200, Ayrshires.

Bloat was determined in most instances by palpation of the left triangular area anterior to the hip and the degree of bloat was indicated by the terms "slight," "marked" or "severe." On the basis of a few instances in which the ruminal pressures were determined either directly with a mercury manometer or indirectly with an instrument devised by Kleiber (unpublished), the pressure in slight bloat was 10 to 23 mm. Hg. and in marked bloat 18 to 40 mm Hg. With one animal in evident distress and consequently designated as severely bloated, the pressure varied from 27 to 34 mm. Mg. In the normal relaxed rumen, the pressure is subatmospheric (2).

The physical condition of the feed and the incidence of bloat. According to our theory (2), bloat or tympany in cattle on green legumes usually results from a lack of sufficient coarse irritating fiber in the diet to elicit the eructation reflex. On the basis of that theory, bloat has been produced by depriving cows on immature alfalfa pasture of coarse roughage and prevented by supplementing such pasture with scabrous feeds such as Sudan grass hay or pasture (3). These results by no means prove the theory, however: conceivably, the presence or absence of coarse scratchy material in the diet of bloated cattle could be a mere coincidence. The present ex-

Received for publication March 6, 1944.

periment was conducted to determine whether the physical condition of the feed does affect the incidence of bloat. Ground alfalfa hay and concentrates were compared with whole alfalfa hay and concentrates. Since the same feeds were used in approximately the same amounts and proportions, any difference in result should depend upon the physical rather than the chemical character of the alfalfa hay. A preliminary note describing the deficiencies associated with a diet of ground hay and concentrates has been published elsewhere (1).

Four lactating dairy cows, secreting 34 to 63 pounds of milk daily, were used. There were two experimental periods. In period 1 the cows received finely ground alfalfa hay plus concentrates. In period 2 the same cows received unground alfalfa hay from the same lot, together with concentrates. Period 1 was preceded by a 6-day preliminary period during which the animals were accustomed to the feed. The hay was ground to overcome the irritating effect of the coarse alfalfa stems upon the mucosa of the rumen.

Two slightly different rations were used in period 1. For the 6-day preliminary period and the first 9 days of the experiment the mixture was made up as follows: ground alfalfa hay prepared in a hammer mill using a No. 6 (6/64″) screen, 60 per cent; rolled barley, 24 per cent; wheat bran, 10 per cent; soybean meal, 5 per cent; and NaCl, 1 per cent. For the following 6 days the mixture consisted of: ground alfalfa prepared as above, 50 per cent; finely ground barley, 39 per cent; wheat bran, 5 per cent; soybean meal, 5 per cent; and NaCl, 1 per cent. This change was made to determine whether a greater amount of concentrates in relation to ground hay would cause a higher incidence of bloat. Three of the four cows bloated on the first ration, and all bloated on the second. For palatability, these rations were moistened with an approximately equal weight of water just before feeding. Each animal received 7 pounds at the time of the morning milking (4:00 A.M.). Between 8:00 and 11:00 A.M. and again between 1:00 and 3:00 P.M., each was given all she would consume. Between 3:00 P.M. and 4:00 A.M. the cows received no food.

In the second period the system had to be modified somewhat, because one cannot feed concentrates and unground hay simultaneously and still maintain a definite proportion between them.

Had the feeding of roughage and concentrates separately been the only variable, bloat should have been more frequent during period 2 than in period 1. The rate of ruminal gas formation varies directly with the amount of feed consumed; further, the most rapid formation occurs during or shortly after eating (2). Since cattle eat concentrates more rapidly than roughage, the rapid ingestion of concentrates fed alone during period 2 favored bloat production. On diets limited to concentrates, bloat frequently occurs (6).

The concentrate mixture in period 2 was identical with that used during the first 9 days of period 1. Each cow received 3 pounds of concentrates at 4:00 A.M. At 8:00 A.M. enough more concentrates were fed to make up about half of the daily allowance. About 8:15 A.M., when the concentrates had been consumed, the animals were given all the unground alfalfa hay they would consume before 11:00 A.M. Between 1:00 and 2:30 P.M. they had enough hay to make their total daily allowance equal or slightly exceed the average individual consumption on the days during period 1 when they bloated. At 2:30 P.M. they were fed enough concentrates to maintain the same proportion of hay and concentrates as on the days when they bloated in period 1. Since cows 759 and 1003 bloated on both mixtures during period 1, the ratio of hay to concentrates for these animals in period 2 was midway between the two proportions. Cows 1028 and 1030 bloated chiefly on the 50 per cent hay–50 per cent concentrate ration; and thus this same ratio was maintained for these animals during period 2. The cows were

TABLE 1

Bloat and daily feed consumption on ground alfalfa hay and concentrates as compared with alfalfa hay without grinding and concentrates

Alfalfa hay	Cow 759		Cow 1003		Cow 1028		Cow 1030	
	Av. feed eaten, pounds	Times bloated*	Av. feed eaten, pounds	Times bloated	Av. feed eaten, pounds	Times bloated	Av. feed eaten, pounds	Times bloated
Ground	32.5	6	42.7	8	28.3	3	29.7	4
Unground	38.5	1	45.5	None	37.0	None	39.9	None

* "Times bloated" refers to the number of days on which bloat occurred.

observed constantly for evidence of bloat between 8:00 A.M. and 12:00 noon and again between 1:00 and 5:00 P.M.

Table 1 shows the incidence of bloat and the average individual feed consumption. Twenty-one cases of slight bloat were encountered while the cows were receiving ground alfalfa, and only one case on whole alfalfa. Often, during period 1, gas under no appreciable pressure could be palpated; but these cases were not designated as bloat. As a rule, bloat occurred during or following the afternoon feeding and persisted 30 to 120 minutes. In four instances when bloat occurred in the morning, the same animals bloated again in the afternoon. This finding agrees with our previous experience in that bloat most often occurred during or after a period of eating, when the rumen was quite full.

One may ask why one case of bloat occurred on whole alfalfa and concentrates. Bloat occurs occasionally when large amounts of concentrates are fed. This animal, a Jersey, was a particularly greedy feeder for her size. She weighed 950 pounds as compared with an average of 1,480 pounds

for the other three animals, which were Holsteins. On the day in question she consumed 4 pounds more than on any day of period 1. On certain days we purposely increased the amount above the average for the days when bloat occurred during period 1. Not only was the whole alfalfa more palatable, but there was also less tendency for the animals to go off feed. Consequently the average consumption in period 2 was 6.9 pounds per day per animal greater than in period 1. Bloat most often occurs after the eating of large amounts; and thus, on the basis of feed consumed, more bloat would have been expected in period 2 than in period 1. It is difficult to escape the conclusion that the physical, rather than the chemical, character of the diet accounts for the greater frequency of bloat during period 1.

Although definite bloat could be produced by feeding ground alfalfa and concentrates, none of the resulting bloat cases was severe enough to require treatment. We cannot satisfactorily explain this fact. That the ground hay provided very little irritation seems certain, since only one or two feeble attempts to ruminate were observed when the cows were on the ground-alfalfa ration. As Schalk and Amadon (7) have shown, coarse roughage provides the stimulus for the regurgitation reflex. The cows ruminated normally when changed to whole alfalfa in period 2. The possibility that the rumen ingesta may have passed on to the omasum too rapidly for fatal bloat to occur is a tentative explanation. Had our studies been more prolonged, fatal bloat might have ensued, for Mead and Goss (6) reported it in cows limited to concentrates. Conceivably, fatal bloat in animals maintained on such diets for long periods may be due to ruminal atony, or it may have been that death was erroneously attributed to bloat. The animals had bloated frequently and were bloated when found dead but they were not actually observed in a state of tympany immediately preceding death.

Bloat on machine-harvested alfalfa tops. In the production of bloat, feeding green tops in the barn has certain advantages over pasturing: (A) The consumption of feed over definite intervals of the day can be more easily determined. (B) If one is interested in the rate of gas production, the feeding of tops to a confined animal has definite advantages. (C) In certain fields it may be impossible to produce bloat by pasturing because of contaminating weeds or grasses. If the weeds are lower than the alfalfa, they may be avoided by cutting the tops and feeding them in the barn. Animals pasturing on such fields, however, will often consume weeds as well as alfalfa; and thus, if the weeds are sufficiently coarse, bloat will be prevented.

For the first 5 days, four lactating cows were used; during the last 5 days, six lactating cows. In a preliminary period of 2 days the cows received grain only. During the first 5 days of the trial the cows received their regular daily allowance of concentrates (6–8 pounds) at 4:00 A.M. and

3:00 P.M.; during the last 5 days they received double their previous amounts of concentrates, fed in equal amounts at 4:00 and 7:45 A.M., 12:30 and 3:00 P.M. The cows had access to all the tops they would consume between 8:00 A.M. and 2:30 P.M. They were stanchioned during the feeding period and were turned out for water for a few minutes once in the morning and again once in the afternoon. For maximum consumption, the tops were fed in small amounts so that the animal received a fresh supply at approximately half-hour intervals. Severely bloated animals were treated with turpentine and deprived of further feed for the day.

The alfalfa tops were cut when the alfalfa was in a very succulent stage, 10 to 14 inches high. We attempted to cut tops about 4 inches long; but because of variations in plant growth they varied from about 2 to 8 inches. The tops were cut with an ordinary mower equipped with a metal apron for

TABLE 2

Bloat on green alfalfa tops: total tops consumed, tops consumed when bloat was first manifest (figures in parenthesis), and maximum bloat reached during the day

Date (1943)	Cow 1003	Cow 760	Cow 1030	Cow 788*
7–27	124 (116) slight	76	118	.....
7–28	64 (61) severe	43	79	.....
7–29	76 (76) severe	53	90 (43) slight	.....
7–30	52	33	75 (41) marked	.....
7–31	52	23	69	.....
8–24	78	40	73	47
8–25	86 (80) severe	86	117	55
8–26	86 (86) severe	69	88 (88) severe	31 (8) slight
8–27	61 (55) severe	71	15	60
8–28	67 (67) severe	44	27	39 (37) slight

* No observations on this animal prior to 8–24.

catching the tops, the outer end of the sickle bar being held at the required height by means of a special shoe. One person followed the mower and raked the tops onto the apron.

Twelve cases of bloat were produced over the 10-day period, seven of which were severe enough to require treatment (table 2). Three cows failed to bloat. Because these three consumed similar amounts of feed, the data on only one (cow 760) are included in the table. The feed consumption of cows 760 and 788 (Jerseys) is considerably less than for the other two animals, which were Holsteins and therefore considerably larger. About one cow out of four bloated each day—that is, only about half the number we have usually encountered while pasturing alfalfa at a similar stage of development. The fact that certain individuals bloat with considerable regularity can be utilized to advantage in certain studies relating to bloat.

That bloat can be produced by feeding green tops is interesting because the feeding of cut green alfalfa in the corral has been successfully employed

in preventing bloat. Bloat is probably avoided by this procedure because the coarse lower stems are included and also because the alfalfa is usually cut at a more advanced stage. The fact that less bloat results from feeding tops than from pasturing may be explained by the possibility that coarser stems are cut than the cow would normally consume while pasturing.

Some tops were cut coincidentally with those fed green and dried in a fruit dehydrater. When these dry tops were fed to three cows over a 4-day period, only two slight cases of bloat occurred. The cows were given all the dry tops they would consume between 7:00 A.M. and 2:30 P.M. At 4:00 A.M., and again at 3:00 P.M. each cow received 4 pounds of concentrates. Drying of the tops apparently reduced their capacity for producing bloat.

TABLE 3

Feeding of barley straw to prevent bloat on alfalfa pasture

Date (Oct., 1943)	Concentrates per cow, pounds	Straw*		Number of cows	Number cases of bloat
		Fed per cow, pounds	Av. amount consumed, pounds		
4	6†	.....	.....	8	4
5	6†	.....	.....	8	5
6	6†	.....	.....	8	5
7	.....	.....	.....	8	3
8	.....	5	1.7	8	4
9	.....	5	2.2	8	4
10	.....	8	5.0	7	3
11	.....	8	4.1	7	4
12	.....	.....	.....	8	6
13	.....	.....	.....	8	5
14	.....	.....	.....	8	6
15	6‡	.....	.....	8	3
16	6‡	.....	.....	8	6

* When 5 pounds of straw were fed per cow, this amount was given 2 hours prior to pasturing on alfalfa; when 8 pounds were given, the cows had access to the straw overnight.

† Concentrates fed afternoon before day of trial.

‡ Concentrates fed on morning of trial rather than night before.

This was not because of lowered feed consumption: the total dry matter ingested was about the same as when green tops were fed. Probably the dry tops were more irritating, but more rumination occurred on both green and dry tops than one would expect if the same field, from which the tops were cut, were to be pastured. At any rate, these preliminary experiments do not indicate that dry tops are satisfactory for producing bloat.

Feeding of barley straw to prevent bloat. Earlier studies (3) indicated that bloat on alfalfa pasture could be prevented by supplementing with Sudan grass hay or pasture. Alfalfa hay varied in effectiveness, presumably according to its coarseness. Since straw is available in most regions at a low cost, its effectiveness in preventing bloat was studied. Four lactating and three or four dry dairy cows were used. All were treated similarly

except that the dry cows were fed straw as a group, whereas the lactating cows were fed individually. Table 3 shows details of the feeding and the frequency of bloat. Obviously, straw did not prevent bloat under the conditions of this experiment. One cow bloated after consuming 5.5 pounds of straw the night before. There may have been a very slight decrease in the incidence of bloat; but three animals bloated severely and required treatment while receiving straw. A tentative explanation of the ineffectiveness of straw is the rather small amount consumed. As the consumption of Sudan grass hay was not measured in our earlier studies (3), we do not know the actual differences in amounts of Sudan grass hay and straw consumed. We are basing our explanation, therefore, on the well-recognized fact that barley straw is rather unpalatable.

TABLE 4

Variations in individual susceptibility to bloat of cows 1003, 760, and 1030, as compared with the incidence of bloat for the group as a whole

Key: D, definitely slightly bloated; M, markedly bloated; S, severely bloated

Date (1943)	Number cows in trial	Number cows bloated	Cow 1003	Cow 760	Cow 1030	Date (1943)	Number cows in trial	Number cows bloated	Cow 1003	Cow 760	Cow 1030
7–27	4	1	D	.....	.....	9–23	10	2	.....	.....	D
7–28	4	1	S	.....	.....	9–24	10	3	D	.....	.....
7–29	4	2	S	.....	D	9–27	10	3	D	.....	.....
7–30	4	1	.....	.....	M	9–30	10	4	M	.....	.....
7–31	4	0	.....	.....	.....	10–1	10	5	D	.....	.....
8–24	6	0	.....	.....	.....	10–2	10	3	.....	.....	.....
8–25	6	1	S	.....	.....	10–3	10	2	.....	.....	.....
8–26	6	3	S	.....	S	10–4	8	4	M	.....	.....
8–27	6	1	S	.....	.....	10–5	8	5	M	.....	.....
8–28	6	2	S	.....	.....	10–6	8	5	M	M	.....
9–13	29	6	.....	.....	.....	10–7	8	3	M	.....	.....
9–14	28	9	D	.....	M	10–8	8	4	M	.....	D
9–15	27	12	M	Out*	M	10–9	8	4	D	.....	D
9–16	28	9	.....	M	D	10–10	7	3	M	.....	.....
9–17	28	11	M	D	D	10–11	7	4	D	.....	D
9–18	13	2	.....	.....	.....	10–12	8	6	D	.....	D
9–19	10	2	.....	D	.....	10–13	8	5	D	M	M
9–20	10	3	S	.....	D	10–14	8	6	D	M	D
9–21	10	4	S	D	.....	10–15	8	3	.....	M	.....
9–22	10	2	.....	.....	.....	10–16	8	6	D	M	D

* "Out" means not in trial on this date.

Variations in individual susceptibility. As already reported (3), individuals appear to differ markedly in susceptibility to bloat. Although more extensive observations have confirmed this belief, clearly too, an individual may vary in susceptibility. In other words, animals hitherto rather resistant suddenly become very susceptible. As examples, data on individual susceptibility of cows 1003, 760, and 1030 are shown in table 4. The number of animals in the trial and the total number bloating each day are also shown

in order that the results may be better evaluated. Cow 1003 bloated more regularly than the other three animals shown in the table. Severe bloat, however, occurred in her most regularly between July 27 and August 28, when the incidence of bloat for all the animals on test was low. Between October 11 and 16 she bloated only slightly even though the incidence of bloat for the group was high. Cows 760 and 1030 were very irregular. Cow 760 did not bloat for 12 days beginning July 27, whereas beginning October 13 she was markedly bloated on four consecutive days. Though some of these variations undoubtedly result from variations in appetite, this explanation does not suffice for all cases. We have data on food consumption for cow 760 during part of both periods mentioned. The average consumption of green tops over the first 2 hours of the feeding period on August 24 to August 28 inclusive was 40 pounds. In addition, this animal received 14 pounds of concentrates per day. Her average consumption for the first 2 hours of the feeding period on October 13 to 16 inclusive was 35 pounds. On October 13 and 14 she received no grain, and on the following 2 days she received 6 pounds shortly before being turned out to pasture. Actually, therefore, she consumed more during the first 2 hours of the period when she did not bloat than during the last period, when she bloated each day. Her grain consumption, too, was greater during this first period. To be sure, we have considered only the amount of alfalfa consumed during the first 2 hours of the feeding period; but in our experience this is usually a reliable criterion in comparing food intake on different days.

If feed consumption does not explain all the variations in individual susceptibility, what is responsible? Although our data give no clue, we suspect that variations in ruminal motility may be involved. As we have pointed out earlier (2), belching occurs only when the rumen is in an active state of contraction. Apparently a lack of coarse roughage for a considerable period may lead to faulty ruminal contractions.

Time at which bloating occurs. In earlier experiments (3), a majority of animals bloated 1.5 to 3.5 hours after being turned onto pasture. Although further experiments conducted under similar conditions have confirmed this result, we now find that the time of bloating varies considerably according to the feeding regime. In the ground hay and concentrate experiment cited above, all but 4 of the 21 cases of bloat occurred after 1:30 P.M. even though feeding began at 4:00 A.M.

Table 5 shows how supplemental feeding affects the interval between the time the cows are turned into alfalfa pasture and the occurrence of bloat. The feeding of grain just before pasturing greatly reduced the interval before bloat was manifest. Our data do not indicate, however, that this procedure increased either the number of cows bloating or the severity of bloat.

Susceptibility of dry and lactating cows. For comparison of susceptibility, 11 dry and 17 lactating cows were turned into the same field. There

were 9 cases of bloat over the 4-day period (20.5 per cent) among the dry cows as compared with 32 cases of bloat for the lactating cows (47.0 per cent). These data are in line with what one would expect on the basis of feed consumption—the lactating cows eating considerably more, as we shall show later. There also appears to be a tendency for lactating cows to bloat more severely.

Amount of green alfalfa consumed. Table 2 shows the consumption of green alfalfa tops in the barn. Studies were also made on the consumption

TABLE 5

The relation of supplemental feeding to the time (in minutes) for bloat to occur after cattle are turned into alfalfa pasture

Date (Oct., 1943)	Supplemental feeding	Cow 597	Cow 738*	Cow 757	Cow 1028	Cow 1046	Cow 788	Cow 760	Cow 1003	Cow 1030	Average
4	Grain afternoon before	.....†	75	.....	65	275	212	.....	85	.....	142
5	Grain afternoon before	.....	.....	430	75	85	75	.....	439	.....	221
6	Grain afternoon before	.....	.....	.....	345	45	65	170	352	.....	195
7	None	.....	331	.....	143	.....	103	.....	331	.....	227
8	Straw night before	.....	.....	.....	140	.....	175	.....	360	285	240
9	Straw night before	.....	153	.....	122	.....	63	.....	326	301	193
10	Straw night before	.....	287	363	115	.....	68	.....	328	.....	232
11	Straw night before	.....	105	350	93	.....	100	.....	410	410	245
12	None	313	.....	.....	71	105	88	.....	388	293	210
13	None	.....	133	.....	93	.....	143	163	333	247	185
14	None	.....	104	.....	58	91	51	73	371	186	133
15	Grain 1 hour before	.....	97	.....	46	.....	71	46	.....	.....	65
16	Grain 1 hour before	140	28	.....	.....	38	28	33	106	106	68

* This cow received no straw at any time, and cow 1028 received no straw on October 10 and 11.

† Blank spaces indicate that no bloat occurred.

of alfalfa during the first 2 hours of pasturing. The cows were weighed before and after pasturing, and all voided urine and feces were collected and weighed. Insensible losses were not taken into account. As the period of pasturing extended from 8:00 A.M. to 10:00 A.M., there were also some variations in dew. In samples taken on one representative day the per cent dry matter of the alfalfa ranged from 15.8 at 8:10 to 19.5 at 10:15 A.M. We are giving these figures because variation in moisture is presumably the major source of error. As the animals from which data are cited were quiet and well trained, there was no appreciable error due to failure in collecting all excreta. We found, however, that very few of the other animals at our

disposal were so gentle as might be desired, particularly for the collection of feces. The animals were allowed to graze when and where they chose in the field; but a halter with a long lead rope restrained them when excreta were being weighed and recorded.

The amount of alfalfa consumed during the first 2 hours of pasturing is shown in table 6 for one dry and one lactating cow under several different regimes of supplemental feeding. The average initial weight before pasturing for the 13 days of the experiment was 824 pounds for cow 757 and

TABLE 6

Amount of green alfalfa consumed by a dry and a lactating cow over a two-hour period and its relation to supplemental feeding

Date (1943)	Supplement fed	Alfalfa consumed, in pounds	
		Cow 757 (dry)	Cow 760 (lactating)
10–7	None	17.0	43.5
10–12	None	19.7	53.8
10–13	None	19.5	50.6*
10–14	None	16.2	30.8†
	Average	18.1	44.7
10–5	6 pounds concentrates the night before	12.4‡	49.6
10–6	6 pounds concentrates the night before	12.5	39.3*
	Average	12.5	44.5
10–15	6 pounds concentrates in morning	15.2	29.2†
10–16	6 pounds concentrates in morning	3.0§	28.8†
	Average	9.1	29.0
10–8	5 pounds straw at 6:30 A.M.	35.2	60.2
10–9	5 pounds straw at 6:30 A.M.	25.8	50.0
10–10	8 pounds straw overnight	22.4‡	36.5
10–11	8 pounds straw overnight	33.8‡	43.6
	Average	29.3	47.6

* Markedly bloated at 11:10 A.M.

† Bloated during more than half of feed consumption period.

‡ Slightly bloated at 3:00 P.M.

§ Ruminal gas could be palpated during all except first 28 minutes of pasturing period, but not under sufficient pressure to call "slight bloat"; this gas no doubt depressed appetite.

909 pounds for cow 760. Assuming that the feed consumption increases in proportion to $\frac{3}{4}$ power of body weight, cow 760 would consume 8 per cent more than cow 757. In comparing feed consumption of lactating and dry cows, one should preferably have several animals in each group. That was our intention, but (as mentioned) it was difficult to find cooperative animals. As an indication of relative capacity, the fat records of these two animals as senior yearlings were 345 pounds for cow 760 and 447 pounds for cow 757. Without supplemental feeding, the lactating animal ate $2\frac{1}{2}$ times more than the other. The feeding of straw just before pasturing increased feed consumption. Although the straw did not prevent bloat, it apparently had a tendency during the first hour or two to prevent the slight accumulation

of gas (really an early stage of bloat) that depresses appetite. Individuals react quite differently to gas accumulation. Many will stop eating the moment gas can be palpated, whereas others will continue eating even though the gas is under considerable pressure. These latter animals are more liable to bloat seriously. The fact that alfalfa consumption is actually stimulated by prior feeding of straw may partly explain why it is not more efficient in reducing the incidence of bloat.

The feeding of concentrates just before pasturing had the opposite effect from feeding straw: the consumption of alfalfa was reduced, largely because bloat depressed appetite. When concentrates were fed the night before pasturing, the feed consumption was comparable with that when no concentrates were fed.[1]

As already mentioned, the rate of consumption of alfalfa by pasturing was comparable with the consumption of green tops in the barn over a 2-hour period. Probably, then, the daily consumption of tops in the barn as shown in table 2 gives a fair index for similar periods of time in the field. We have data on one dry cow pastured 6 hours daily for 3 days. Her average initial weight before pasturing was 1,079 pounds, and she consumed an average of 33.7 pounds daily over the 6-hour period. This cow received no supplemental feeding. Comparing this figure with the figures for cow 757 (table 6), which received no supplements, one could estimate that a dry cow eats as much during the first 2 hours as during the next 4. Since, however, different cows and different fields are involved, the estimate is of doubtful value.

A point of primary interest here is the amount of alfalfa consumed before bloating occurs. Table 2 gives some data when green alfalfa tops were fed. As a rule, bloating did not occur until more than 50 pounds had been consumed; but one animal did bloat after consuming 8 pounds. Table 6 gives limited data for cows on pasture. If grain is fed just before pasturing, bloat may take place before much alfalfa is consumed. The variable time at which bloat occurs after cows are turned into pasture (table 5) indicates that there is considerable latitude in the amount consumed before bloating. Assuming that the period of time on pasture before bloat occurs is an index of consumption, there are marked individual differences in this respect. For example, compare cows 788 and 1003 in table 5. The former usually bloated before the end of the second hour of pasturing, whereas No. 1003 usually did not bloat until after the fifth hour even though she was a particularly good feeder (see tables 1 and 2 for feed-consumption data).

[1] We have not ascertained how the feeding of concentrates before pasturing affects the incidence of bloat. Without submitting data in confirmation, Espe, Jacobson, and Cannon (4) state, "Feeding grain, dry hay or silage before putting cattle on legume pastures will help prevent over-eating and, in turn, bloat." The amounts of grain we have used have had no appreciable effect on the incidence of bloat (table 3).

DISCUSSION

These studies support the view that the lack of sufficient coarse irritating material in the diet is the chief cause of bloat on legume pastures. The work on ground alfalfa hay gives clear-cut evidence that the physical character of the feed is an important etiological factor in bloat. Additional evidence is provided by the fact that we have been able to predict bloat on eight different fields, some of them on two or three occasions, merely by taking into account the succulence of the alfalfa and the absence of weeds or grasses. We did fail to produce bloat in one field early in the spring even though we were successful in two other attempts. When pastured early, this field was contaminated with annual grasses. Since the grasses were lower than the alfalfa, we expected that the cows would eat only the alfalfa. On the contrary, they consumed grasses for the most part.

The evidence indicates, further, that lactating cows are more subject to bloat because of greater feed consumption. Reviewing the evidence submitted in table 2, however, one sees that bloat is not invariably associated with high feed consumption. Because of variations in individual susceptibility, not correlated with feed consumption or with the bloat-producing capacity of the feed, these appear not to be the only factors that determine whether or not a certain individual will bloat on a given day. As we have already suggested, variations in ruminal motility may account for these changes in susceptibility.

Bloat has been produced by feeding ground hay and concentrates or by feeding alfalfa tops in the barn as well as by pasturing.[2] We can add somewhat to our previous discussion (3) on means of inducing bloat by pasturing legumes. Very young alfalfa (4–6″) for instance, is rather unpalatable and thus not suitable for bloat studies. Cattle turned into young alfalfa will invariably consume the less succulent alfalfa (more stemmy) on the ridges used to check irrigation water. On the other hand, alfalfa is no longer suitable for bloat studies when the top part of the plant becomes stemmy. Apparently one reason why bloat is produced rather easily under our conditions is that we can supply the alfalfa with optimal amounts of water throughout the summer. In late fall a field may remain in a bloat-producing stage for several weeks because of the slow maturity resulting from cooler weather.

SUMMARY AND CONCLUSIONS

1. In a series of experiments involving an average of 9 cows for 61 days, 194 cases of bloat were produced.

2. Demonstrating that the physical character of the feed is an important etiological factor in bloat, 21 mild cases of bloat were produced in 4 cows over

[2] The effect of feeding green alfalfa tops to ewes in the barn has also been studied (unpublished data). Over an 11-day period 26 cases of bloat were encountered in 5 ewes; but none of the cases was severe enough to require treatment.

a 15-day period by feeding ground alfalfa hay and concentrates in a 60–40 or 50–50 ratio. Only one case of bloat occurred when concentrates were fed in the same proportion with the same hay unground.

3. Twelve cases of bloat, 7 of which were severe enough to require treatment, were induced by feeding green alfalfa tops in the barn to 4–6 cows for 9 days.

4. Supplemental feeding of barley straw at night to cows pastured on alfalfa during the day was not effective in preventing bloat.

5. Variations in individual susceptibility occurred that could not be explained either by feed consumption or by the nature of the feed. We conclude that these changes in susceptibility of an individual depend on some change in physiological activity of the rumen—for example, motility.

6. Bloat occurred sooner after animals were turned into pasture if grain was fed just before pasturing.

7. Certain data indicate that lactating cows are more susceptible than dry cows.

8. Over a 12-day period, the average amount of alfalfa consumed by a dry cow during the first two hours of pasturing was 19.4 pounds as compared with 43 pounds for a lactating cow. The feeding of straw overnight, before pasturing, markedly increased the consumption of alfalfa by the dry cow during the first two hours; the affect on the lactating cow was less conspicuous. Feeding of concentrates just before pasturing decreased consumption of alfalfa, with no apparent effect upon the incidence or severity of bloat.

REFERENCES

(1) Cole, H. H., and Mead, S. W. A Physical Deficiency in the Ration of Ruminants. Science, 98: 543. 1943.

(2) Cole, H. H., Mead, S. W., and Kleiber, Max. Bloat in Cattle. Calif. Agr. Expt. Sta. Bul. 662. 1942.

(3) Cole, H. H., Mead, S. W., and Regan, W. M. Production and Prevention of Bloat in Cattle on Alfalfa Pasture. Jour. Anim. Sci., 2: 285. 1943.

(4) Espe, D., Jacobson, N. L., and Cannon, C. Y. Why Do Cows Bloat? Holstein-Friesian World, 40: 1020. 1943.

(5) Kleiber, Max, Cole, H. H., and Mead, S. W. Bloat in Cattle and Composition of Rumen Gases. Jour. Dairy Sci., 26: 929. 1943.

(6) Mead, S. W., and Goss, H. Some Results of Eight Years of Investigation Concerning the Role of Roughage in the Diet of Ruminants. Jour. Dairy Sci., 19: 465. (Abstract.) 1936.

(7) Schalk, A. F., and Amadon, R. S. Physiology of the Ruminant Stomach (Bovine). Study of the Dynamic Factors. N. Dak. Agr. Expt. Sta. Bul. 216. 1928.

NON-PERMEABILITY OF THE MAMMARY GLAND TO THYROID HORMONE

E. P. REINEKE AND C. W. TURNER*
Missouri Agricultural Experiment Station, Columbia

As a result of experiments first reported by Graham (10, 11) and repeatedly confirmed by a number of investigators, it is now well established that increases in both the milk yield and butterfat test of lactating cows can be brought about by the administration of thyroid substance or thyroxine. Practical use of thyroid for this purpose was prevented by its limited supply and high cost.

However, detailed studies of the authors on the formation of thyroidally active compounds by combining iodine with casein (25, 18, 20, 26) finally resulted in the development of an inexpensive process for the formation of a synthetic "thyroprotein" that exerts many times the effect of U.S.P. thyroid, as measured by the usual assay methods. Proof that the active principal in this synthetic thyroprotein is identical with that in natural thyroid was provided by the recovery in high yield of crystalline d,l-thyroxine (21) and, finally, crystalline l-thyroxine (22).

Pronounced increases in daily milk yield and milk fat percentage were observed when synthetic thyroprotein was fed to lactating cows, as reported by Reineke and Turner (23) and Reineke (19), and confirmed by Van Landingham, Henderson and Weakly (34), and Blaxter (3).

Although much more extensive experiments will be required, particularly on the effects of continuous feeding of thyroprotein to dairy cattle, before its effects can be fully evaluated this product holds considerable promise as a means of increasing and maintaining milk production.

The question then arises whether or not the feeding of thyroprotein would result in transmission of thyroxine into the milk.

Although a few experiments have been interpreted to indicate the passage of thyroid hormone into the milk, no direct evidence in support of such a view has been reported. Having noted that experimental hyperthyreosis reduced the growth rate of rats, Lukacs (16) administered thyroid to lactating rats and observed the growth rate of the young. He reported that the young of the hyperthyroid females were retarded in their growth 31 to 34 per cent. From this he concluded that thyroid hormone passes into the milk and further that the amount present changes according to the concentration in the mother's system. However, it is known that both excessive hyperthyreosis (Herman, Graham and Turner, 13) and thyroidectomy (Folley, 8,

Received for publication March 11, 1944.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 942.

Preheim, 17) will depress the rate of milk secretion. Preheim has presented a good review of the literature.

It appears from an examination of all the experiments in this field that lactation is suppressed by hypothyroidism, stimulated increasingly by higher levels of thyroid secretion, and is again depressed when the stimulation reaches excessive levels. Since both thyroidectomy and hyperthyreosis depress milk secretion, the growth rate of nursing young is too nonspecific to determine whether or not thyroprotein is transmitted to the milk.

Konsulov (15) reported that thyroidin administered to a lactating mother passed into the milk and caused an increase in the carbon dioxide production of the nursing infant. This work must be interpreted with caution, however, since Brody (4) found that the metabolism of rats receiving larger amounts of milk (by reducing the number in a litter) have a markedly higher rate of oxygen consumption than those receiving less milk. Thus following thyroid administration, a higher rate of milk secretion would increase the metabolism of the young due to greater intake of nutrients, without postulating the passage of thyroid into the milk.

In all cases where a more critical and specific measure of the possible transference of thyroid hormone from the maternal system to the milk has been made, the results have been completely negative. Simpson (31) compared the growth rate of twin lambs and kids, one member of each pair being thyroidectomized at about one month of age and the other serving as a normal control. The thyroidectomized animals soon showed symptoms of thyroid deficiency even though the food consisted entirely of the mother's milk for some weeks. He concluded that these facts do not support the idea that a thyroid hormone is present in mother's milk, at any rate in quantity sufficient to supply the needs of the athyroid offspring.

Dorff (6) made similar observations in man in the case of twins, one of which was cretinous. Previous to this, Herman (14) and Gordon (9) noted cases of cretinism which were not improved by nursing, but responded to thyroid treatment. Siegert (30) reported that in cretins with a complete absence of the thyroid gland signs of deficiency may be apparent at birth or shortly thereafter.

In the experiments of Reineke and Turner (24) with thyroidectomized kids and Brody and Frankenbach (5) with thyroidectomized calves all of the thyroidectomized animals rapidly developed cretinism even though they were fed milk from normal animals with intact thyroids.

Earlier experiments in which young mammals were thyroidectomized and continued on normal milk without curative effect on the induced cretinism were reported by Simpson (32) on sheep, Basinger (1) on rabbits, Binswanger (2) on dogs and Salmon (28) on rats.

These extensive experiments on a long series of mammals from the rat to the cow uniformly fail to provide evidence of the transfer of thyroid hormone to the milk of animals with a normal thyroid gland.

It is also reported by Elmer and Rychlik (7) that milk yields only a trace of thyroxine-like fraction after hydrolysis. In his book, "The Endocrine Function of Iodine," Salter (29) reviewed the evidence on the transference of thyroxine through the milk and concluded that "if present at all it must be exceedingly small."

Even though the evidence fails to show a single instance for the passage of normal thyroid secretion into the milk the possibility was suggested that when thyroidal substances are fed continuously the thyroid level of the blood might be elevated sufficiently to permit passage of traces of active substance into the milk. In view of the possible use of the new synthetic thyroprotein for the stimulation of increased lactation, and the ultimate consumption of the milk so produced by humans, it seemed desirable to investigate further the question whether thyroprotein-fed cows would secrete detectable amounts of this hormone in their milk.

EXPERIMENTAL

Three experiments were conducted in which synthetic thyroprotein was fed to cows, and the milk was fed to suitable test animals for detection of possible thyroidal properties. The synthetic thyroprotein, hereafter designated as "Protamone," was kindly supplied by Dr. W. R. Graham, Jr., Cerophyl Laboratories, Kansas City, Missouri. In experiments number I and II the experimental milk was fed to guinea pigs and in experiment III young thyroidectomized goats were used as test animals. In experiment IV similar milk was tested in man.

Experiments with guinea pigs. Two groups of six mature guinea pigs, paired as to body weight and general condition, were selected for experiment I. Each animal was numbered and placed in an individual cage and as a preliminary, both groups were fed normal pasteurized milk for a period of two weeks to accustom them to this type of diet. Each animal was given 100 ml. of milk daily, and the amount refused was measured back, the actual milk consumption thus being estimated by difference. To provide additional nutritive factors each animal was given 6 grams of dehydrated and finely ground cereal grass[1] daily which was mixed with 25 ml. of milk. A mineral supplement consisting of 1 mg. of iron, as ferric pyro-phosphate; 0.1 mg. of copper, as copper sulfate; and 0.1 mg. of manganese, as manganese sulfate, was fed daily in a gelatin capsule. In addition to the milk-consumption data, the body weight of each animal was recorded daily. One animal in each group failed to do well after 10 days on the normal milk diet so new animals were substituted for each.

Metabolism measurements of the individual guinea pigs on the experiment were made during the last three days of the preliminary period and

[1] Cerogras, kindly supplied by the Cerophyl Laboratories, Inc., 2438 Broadway, Kansas City, Missouri.

at intervals for fourteen days after being placed on the milk from the thyroprotein-fed cow. The determinations were made in a modified Haldane type, open circuit respiration apparatus for small animals, using the weight of carbon dioxide produced during a period of one hour as an indirect measure of the metabolism. To facilitate comparisons of values for individual animals the data were converted to the basis of carbon dioxide production per 100 gm. body weight per hour.

After 14 days on the milk diet, raw milk from normal Holsteins and from Holsteins on a high dosage of thyroprotein was substituted in the diets of groups I and II, respectively.

Rather extensive feeding trials with synthetic thyroprotein of the potency now available (3 to 4 per cent as potent as d,l-thyroxine) have shown that 1 to 1½ grams per 100 lbs. body weight daily is tolerated nicely by cows and will cause significant increases in their production of milk and butterfat. In order to make the present test as critical as possible, the cow supplying the milk for this experiment was given the maximum amount of thyroprotein that she would tolerate. Beginning on March 18, 1943, she was fed 90 grams of thyroprotein daily in her grain feed, an amount far above what would ever be used in practice. By April 7, it was evident from the cow's physical symptoms that she would not tolerate this dosage indefinitely. Accordingly the dosage was reduced to 45 grams daily, and continued at this level to the end of the experiment. The guinea pigs in group II received milk from this cow from April 1 to April 14, 1943.

RESULTS

Inasmuch as there were no significant differences in the milk consumption or trend of body weight the detailed data on these factors will be omitted for the sake of brevity. After the guinea pigs had become accustomed to the milk diet they consumed an average of 80 to 90 ml. of fluid milk daily. In addition they received approximately 25 ml. daily with the grass supplement, making a total consumption of 100 ml. or more daily.

After an initial drop in body weight during the preliminary period while the animals were becoming accustomed to the milk diet there was some gain in weight, and then the values leveled out to a uniform figure. The milk from the "Protamone-fed" cow had no effect on body weight of guinea pigs as compared to the controls on normal milk.

A comparison of the average carbon dioxide output of the two groups of guinea pigs, determined at intervals during the experiment, is shown graphically in figure 1. With the exception of the minor fluctuations that are always encountered in metabolism studies, the average values for both groups remained at practically the same level throughout the experiment. Therefore, no evidence could be found for the passage of thyroidal substance into the milk of "Protamone-fed" cows during the period covered in this experiment.

All of the earlier clinical and experimental work on thyroid administration indicates that the dosage does not become cumulative over a long period of time. Instead there is a gradual rise in metabolism, starting about the third day after dosage is begun, and reaching a peak in about two weeks. The metabolism then remains constant at this new level as long as the dosage is continued. Nevertheless, it seemed desirable to extend the experiments on milk from "Protamone-fed" cows over a longer period to determine whether any hormone would be transmitted to the milk with continuous feeding.

Therefore, experiment II was set up using the same experimental procedure described for experiment I. However, the period during which the experimental milk was fed was extended to 8 weeks. During the last 2

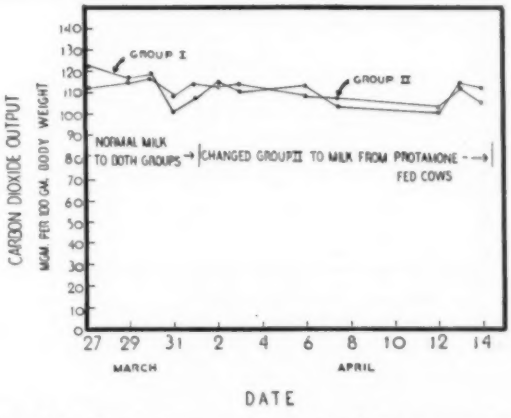

CARBON DIOXIDE OUTPUT
MGM PER 100 GM. BODY WEIGHT
GROUP I
GROUP II
NORMAL MILK TO BOTH GROUPS
CHANGED GROUP II TO MILK FROM PROTAMONE FED COWS
27 29 31 2 4 6 8 10 12 14
MARCH
APRIL
DATE

FIG. 1. The effect of milk from a "Protamone-fed" cow on the resting carbon dioxide output of mature guinea pigs. Group I received normal milk throughout. During the last two weeks group II received milk from a cow that was being fed a high dosage of "Protamone."

weeks the animals in group I (receiving milk from a "Protamone-fed" cow) were injected with l-thyroxine at the rate of 2.5 micrograms per 100 gm. body weight daily. Group II received normal Holstein milk throughout. Milk for the experimental group was obtained from a Holstein cow receiving the more nearly optimal dosage of 1½ gm. "Protamone" per 100 lbs. body weight. On this dosage this cow showed an initial rise of 11 per cent in milk production, but no serious losses in body weight. Two groups of 6 guinea pigs were started on experiment, but 1 animal of each group failed to become adapted to the milk diet, and, therefore, data are reported on 5 animals in each group.

The milk consumption and body weights of both groups followed the same trend as described in the previous experiments. Therefore, no further comment will be made on these points.

A comparison of the average carbon dioxide output of the two groups of guinea pigs is shown graphically in figure 2. Here again both groups follow the same metabolic trend with minor deviations, throughout the six weeks on the experimental milk diet. Analysis of the individual determinations during the six-week period before group I was injected with thyroxine revealed that 95 determinations of the carbon dioxide output of the animals in group I averaged 106.5 mg. per 100 gm. body weight, standard deviation 11.803 mg. The average carbon dioxide output in 88 determinations on group II during the same period was 102.8 mg. per 100 gm. body weight, standard deviation 14.051 mg. The standard error of the difference was 6.0676 mg., t value, 0.576. As shown by the "t" test (Snedecor, 33) the difference between the two groups is insignificant. By comparison with

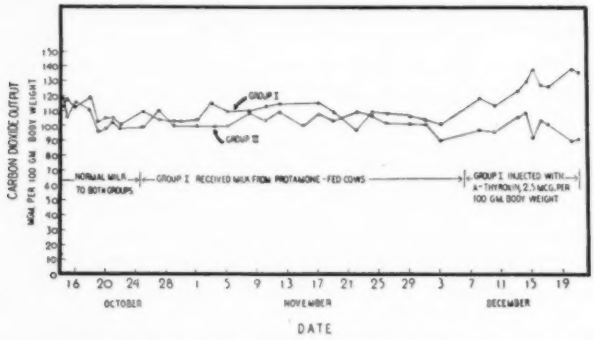

CARBON DIOXIDE OUTPUT
MGM. PER 100 GM. BODY WEIGHT
GROUP I
GROUP II
NORMAL MILK TO BOTH GROUPS
GROUP I RECEIVED MILK FROM PROTAMONE-FED COWS
GROUP I INJECTED WITH l-THYROXIN 2.5 MCG. PER 100 GM. BODY WEIGHT
16 20 24 28 1 5 9 13 17 21 25 29 3 7 11 15 19
OCTOBER
NOVEMBER
DECEMBER
DATE

FIG. 2. The effect of milk from a "Protamone-fed" cow on the resting carbon dioxide output of mature guinea pigs. During the six-week period that group I received the experimental milk no significant elevation of metabolism was observed. Injection of 2.5 micrograms of l-thyroxine during the final period caused a 25 per cent increase in metabolic rate.

these values obtained on a milk diet, 110 determinations of the resting carbon dioxide of normal guinea pigs on a stock diet as determined previously (unpublished) was 105.7 mg. per 100 gm. per hour. Thus it is apparent that the guinea pigs fed the experimental milk in the maximum amount they would consume did not differ significantly in their metabolism from similar animals on a normal milk diet, or on a stock diet. No evidence could be found, therefore, for the transmission of thyroidal substance to the milk of cows fed "Protamone."

It is well known that the guinea pig is the most responsive to thyroidal stimulation of all the laboratory animals, and, therefore, should be capable of detecting such transference if it occurs in biologically significant amounts. As an indication of their sensitivity, attention is drawn to the results obtained (fig. 2) when 2.5 micrograms of l-thyroxine per 100 gm. body weight was injected daily in the animals of group I. The average carbon dioxide

output of this group for six days previous to the injections was 105.4 mg. per 100 gm. body weight. The carbon dioxide output began to rise by the third day of injection and reached the maximum by about the eighth day. The carbon dioxide output during the last six days on which determinations were made was 132.5 mg. per 100 gm. body weight, an increase of 25.7 per cent.

Experiment with thyroidectomized goats. In addition to the experiments with guinea pigs, milk from "Protamone-fed" cows was fed to a group of thyroidectomized goats in order to test possible thyroidal effects.

Thyroidectomized animals are very sensitive to thyroidal substance since

TABLE 1

The metabolism of thyroidectomized goats before and after receiving milk from thyroprotein-fed cows

Date	Metabolism, calories per day					Remarks
	Goat No. 40	Goat No. 26	Goat No. 57	Goat No. 19	Average all goats	
3/1/43	727	1024	936	947		Preliminary period
3/2/43	669	1026	982	837		
3/3/43	808	1007	852	1018		
3/4/43	882	926	806	806		
3/5/43	806	1192	1236	994		
Mean	778	1035	962	920	924	
3/9/43	.....	1247	1312	1010		Each goat consumed 5 lbs. milk from thyroprotein-fed cows daily
3/10/43	969	1245	1068	958		
3/11/43	985	1219	743	942		
3/12/43	619	716	1184	814		
3/16/43	773	1002	926	1144		
3/18/43	587	1119	909	798		
3/19/43	811	811	689	1066		
3/20/43	703	714	781	1284		
3/22/43	766	879	788	676		
Mean	777	995	933	966	918	

they have a greatly reduced metabolism, and also show pronounced physical symptoms of deficiency that can be corrected only by thyroidal hormone.

For the experiment to be reported, four thyroidectomized goats with long-standing thyroid deficiency were used. These animals were thyroidectomized shortly after birth in the spring of 1941. Shortly after thyroidectomy, they were given sufficient thyroprotein to maintain them in a healthy condition, this treatment being continued until March 21, 1942. At this time the thyroprotein therapy was stopped completely and a hypothyroid condition allowed to develop.

The goats were trained for measurements of oxygen consumption by the spirometer method. During a preliminary period from March 1 to March 5, 1943, measurements of their resting oxygen consumption were made daily. They were then given 5 lbs. of milk daily per goat from 2 Jersey cows that

were receiving 20 grams of thyroprotein daily in their feed. The milk diet was started on March 5 and stopped on March 19. Frequent measurements of metabolism were made during this period. Since thyroid hormone, if present, is known to exert its effect for a considerable time, the determinations were continued to March 22.

The results are given in table I, the data having been converted to the basis of metabolism in calories per day.

During the preliminary period, the average metabolism was 924 calories per day. During 9 days after the experimental milk was fed, the average value was 918 calories per day. Although fairly large day to day variations in metabolism of individual goats were observed, these are no greater than are encountered normally. No definite trend toward a higher metabolic level due to feeding the milk from thyroprotein-fed cows is apparent in the records of any of the animals.

Of at least equal significance is the fact that no evidence of correction of the thyroid deficiency symptoms of the goats was observed during the milk-feeding period. Prior to starting this trial, goats No. 26 and 40 in particular showed extreme symptoms of hypothyroidism. They were very weak, dull-looking, paunchy, and sluggish in their movements and general appearance. The hair was dull and coarse and the skin thick and scaly. No improvement was noted while the milk was fed.

At the close of the experiment each goat was given $\frac{1}{4}$ gram of synthetic thyroprotein daily for a period of two weeks. Immediate improvement was noted. Within a week the goats became stronger, more alert, less paunchy, and their appetites improved. The coarse dull hair and outer layers of the thick, scaly hide were shed, followed by growth of a new, finer coat of hair.

On the basis of these results, it must be concluded that no evidence of transmission of thyroid hormone into the milk could be detected, either quantitatively by measurement of the metabolism of the milk fed test animals, or qualitatively by improvement of their cretinous condition.

Experiments with man. Finally an experiment was set up to determine the influence of milk from "Protamone-fed" cows upon the basal metabolic rate in man. The metabolism was determined by means of a Benedict-Roth Recording metabolism apparatus. The records were taken in the morning immediately upon arising. During the initial period, one quart of normal cow's milk was consumed daily. This period was followed by a period during which milk was consumed from cows fed "Protamone." Following these periods, "Protamone" was taken orally in amounts sufficient to definitely increase the metabolism. Then all thyroidal treatment was discontinued.

Subject I. A girl 17 years old weighing 120 lbs. showed an average B.M.R. of – 2.33 per cent during a period of 16 days in which time one quart daily of normal herd milk was consumed. She then switched to milk from

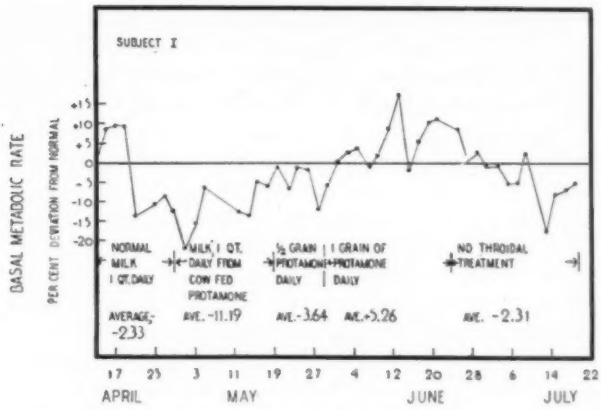


FIG. 3. The effect of milk from "Protamone-fed" cows on the basal metabolic rate in man. During ingestion of 1 quart daily of experimental milk by a 17-year-old girl the basal metabolic rate remained within her usual limits. Administration of "Protamone" directly caused a significant rise in metabolism.

cattle receiving the recommended dosage of "Protamone." Results of the metabolism determinations are given in figure 3. During a period of 20 days on milk from "Protamone-fed" cows her average B.M.R. was −11.19 per cent. For a period of 10 days, she next received daily ½ grain of "Protamone" orally in a gelatin capsule. As her B.M.R. still continued below normal (ave. −3.64%), the amount of "Protamone" was raised to 1 grain

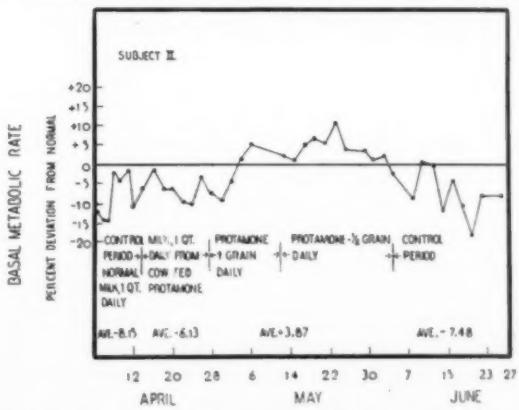


FIG. 4. The effect of milk from "Protamone-fed" cows on the basal metabolic rate in man. One quart of milk from "Protamone-fed" cows taken daily by a 46-year-old man caused no significant deviation from the subject's usual metabolic level. Oral administration of "Protamone" caused a rapid and significant rise in the basal metabolic rate.

daily. On this amount there was an appreciable rise in energy metabolism passing to positive readings in 4 days. The average B.M.R. of the 24-day period was +5.26. Upon the withdrawal of "Protamone," the metabolism began to decline again and during the month the average B.M.R. was −2.31. It is clear from this record that the consumption of one quart of milk per day from cattle fed "Protamone" had no effect upon energy metabolism.

Subject II. A man 46 years old weighing 192 lbs. showed a B.M.R. of −8.15 during a period of 10 days during which one quart of milk from normal cows was consumed daily. During the next 2 weeks 1 quart of milk was consumed daily from cows fed "Protamone." The B.M.R. averaged −6.13, being in the same range as before. However, upon taking 1 grain daily of "Protamone," the metabolism began to increase immediately reaching a first peak in 6 days. The "Protamone" dosage was then reduced to ½ grain and continued for 23 days during which time the average B.M.R. was +3.87. Upon stopping "Protamone" the B.M.R. declined rapidly. These results are shown graphically in figure 4. It is obvious that the consumption of one quart of milk daily from cows being fed "Protamone" had no effect on the B.M.R.; ½ grain of "Protamone," however, was effective in increasing energy metabolism.

DISCUSSION

No evidence could be found for the transmission of thyroxine into milk, as determined by biological tests, even though the cows producing the milk were fed considerable amounts of thyroactive material.

Whether or not such transference could be found would depend upon such factors as (a) the permeability of the milk secreting cells to thyroid hormone, (b) the effective level of hormone in the blood stream, and (c) the sensitivity of test methods for detecting the hormone in the milk.

With regard to the first factor, it is known that the mammary gland is readily permeable to simple iodine-containing compounds such as iodides, since feeding of iodide or iodine-rich feeds is reflected almost at once by an increase in the iodine content of the milk. In the case of "Protamone," however, the active iodine is in firm combination in the thyroxine molecule, which in turn is combined as a constituent amino acid of the iodinated casein. Subsequent to digestion and absorption, it is not entirely established in what form the thyroxine molecule would be combined. It is known that thyroxine is stored in the thyroid as a constituent amino acid of thyroglobulin, a giant protein molecule with a molecular weight of approximately 700,000. It is believed that the circulating hormone is similar to thyroglobulin if not identical with it. Even though evidence has been reported by Graham *et al.* (12) and Reineke *et al.* (27) that the mammary gland is permeable to certain fractions of the serum globulins, it appears extremely unlikely that the milk-secreting cells would allow passage of a huge molecule such as thyroglobulin.

The dosage of "Protamone" employed in at least one of these cases was far above what would ever be used in practice for stimulating increased milk and milk fat production. Therefore, the level of hormone in the blood should certainly have been high enough to test its possible transference under practical conditions.

Finally, as to the sensitivity of the tests employed, it has been repeatedly observed that guinea pigs are among the most sensitive of the laboratory animals to thyroidal stimulation. If physiologically significant amounts of thyroidal hormone had appeared in the milk it should have been detected under the conditions of these experiments.

Therefore, it is concluded that the mammary gland does not permit passage of biologically detectable amounts of thyroidal hormone, when "Protamone" is given as a portion of the diet.

SUMMARY AND CONCLUSIONS

In view of the recent development of highly active synthetic thyroprotein (Protamone) and the possible use of this product for stimulation of increased lactation in dairy cattle, a series of experiments were conducted to determine whether cows fed "Protamone" would transmit any thyroidal substance in their milk.

In one experiment guinea pigs were given approximately 100 ml. of milk daily for two weeks from cows receiving massive doses of "Protamone." In a second trial with guinea pigs, milk from cows receiving a moderate dose of "Protamone" was fed for six weeks. Frequent determinations of metabolism during both experiments failed to show any significant differences between these animals and paired controls receiving a normal milk diet, or normal guinea pigs on a stock diet. Responsiveness of the guinea pigs to thyroidal stimulation was shown by the fact that the experimental group while still on the milk diet showed a 25 per cent increase in metabolism when 2.5 micrograms of l-thyroxine was injected daily for two weeks.

In a similar experiment with thyroidectomized goats no metabolic stimulation or subjective improvement of the cretinism could be detected as a result of feeding milk from "Protamone-fed" cows for two weeks. These animals responded markedly, however, when $\frac{1}{4}$ gram of "Protamone" daily was fed directly.

No elevation of the basal metabolism could be detected when a quart of milk daily from "Protamone-fed" cows was consumed by two human subjects. Significant elevation of the basal metabolic rate occurred when $\frac{1}{2}$ to 1 grain of "Protamone" daily was given orally.

On the basis of these experiments it was concluded that the mammary gland does not permit passage of biologically detectable amounts of thyroidal hormone when "Protamone" is fed to lactating cows.

REFERENCES

(1) BASINGER, H. R. The Control of Experimental Cretinism. Arch. Int. Med., **17**: 260. 1916.

(2) BINSWANGER, F. Studien zur Physiologie der Schilddrüse. III. Schilddrüse und Wachstum. Endokrinologie, **17**: 22. 1936.

(3) BLAXTER, K. L. Stimulation of the Milk Production of Dairy Cows by Feeding Thyroid-Active Iodinated Proteins. Nature, **152**: 751. 1943.

(4) BRODY, E. Litter Size, Growth Rate and Heat Production of Suckling Rats. Amer. Jour. Physiol., **138**: 180. 1942.

(5) BRODY, S., AND FRANKENBACH, R. F. Metabolism and Cardio-Respiratory Activities of Thyroidectomized Cattle. Mo. Agr. Expt. Sta. Bul. 349. 1942.

(6) DORFF, G. B. Sporadic Cretinism in One of Twins, Report of Cases with Roentgen Demonstration of Osseous Changes That Occurred in Utero. Amer. Jour. Dis. Children, **48**: 1316. 1934.

(7) ELMER, A. W., AND RYCHLIK, W. L. Recherches comparatives sur l'excretion d'iode par l'urine, apre's ingestion d'iode mineral, de thyroxine et de diiodotyrosine. Compt. Rend. Soc. de biol., **115**: 1719. 1934.

(8) FOLLEY, S. J. Experiments on the Relation Between the Thyroid Gland and Lactation in the Rat. Jour. Physiol., **93**: 401. 1938.

(9) GORDON, M. B. Childhood Myxedema or So-Called Sporadic Cretinism in North America. Endocrinology, **6**: 234. 1922.

(10) GRAHAM, JR., W. R. The Effect of Thyroidectomy and Thyroid Feeding on Milk Secretion and Milk Fat Production of Cows. Jour. Nutr., **7**: 407. 1934.

(11) GRAHAM, JR., W. R. The Action of Thyroxine on the Milk and Milk Fat Production of Cows. Biochem. Jour., **38**: 1368. 1934.

(12) GRAHAM, JR., W. R., PETERSON, V. E., HOUCHIN, O. B., AND TURNER, C. W. The Utilization of Fractions of the Nitrogen Partition of the Blood by the Active Mammary Gland. Jour. Biol. Chem., **122**: 275. 1938.

(13) HERMAN, H. A., GRAHAM, JR., W. R., AND TURNER, C. W. The Effect of Thyroxine on Milk and Fat Production. JOUR. DAIRY SCI., **20**: 412. 1937.

(14) HERMANN, C. Sporadic Cretinism: Clinical Study of 41 Cases. N. Y. State Jour. Med., **14**: 394. 1914.

(15) KONSULOV, S. Passage of Hormones into Milk. Endocrinology, **16**: 237. 1935.

(16) LUKACS, J. Does the Hormone of the Thyroid Gland Pass into the Mother's Milk. Magyar Orvosi Arch., **32**: 147. 1931.

(17) PREHEIM, DELBERT V. Studies on Thyroidectomized Rats with Special Reference to Lactation and Growth. Endocrinology, **27**: 494. 1940.

(18) REINEKE, E. P. The Chemical Formation of Highly Active Thyroprotein. JOUR. DAIRY SCI., **25**: 702. 1942.

(19) REINEKE, E. P. Practical Trials on the Use of Synthetic Thyroprotein for Increased Production of Milk and Butterfat. JOUR. DAIRY SCI., **26**: 750. 1943.

(20) REINEKE, E. P, AND TURNER, C. W. Formation in vitro of Highly Active Thyroproteins, Their Biological Assay and Practical Use. Mo. Agr. Expt. Sta. Res. Bul. 355. 1942.

(21) REINEKE, E. P., AND TURNER, C. W. The Recovery of Crystalline Thyroxine from Iodinated Casein. Jour. Biol. Chem., **149**: 555. 1943.

(22) REINEKE, E. P., AND TURNER, C. W. The Recovery of L-Thyroxine from Iodinated Casein by Direct Hydrolysis with Acid. Jour. Biol. Chem., **149**: 563. 1943.

(23) REINEKE, E. P., AND TURNER, C. W. Increased Milk and Milk Fat Production Following the Feeding of Artificially Formed Thyroprotein (Thyrolactin). JOUR. DAIRY SCI., **25**: 393. 1942.

(24) REINEKE, E. P., AND TURNER, C. W. Growth Response of Thyroidectomized Goats to Artificially Formed Thyroprotein. Endocrinology, 29: 667. 1941.

(25) REINEKE, E. P., WILLIAMSON, M. B., AND TURNER, C. W. The Effect of Progressive Iodination on the Thyroidal Activity of Iodinated Casein. Jour. Biol. Chem., 143: 285. 1942.

(26) REINEKE, E. P., WILLIAMSON, M. B., AND TURNER, C. W. The Effect of Progressive Iodination Followed by Incubation at High Temperature on the Thyroidal Activity of Iodinated Proteins. Jour. Biol. Chem., 147: 115. 1943.

(27) REINEKE, E. P., WILLIAMSON, M. B., AND TURNER, C. W. Utilization of Glycoprotein of the Blood Plasma by the Lactating Mammary Gland. Jour. Biol. Chem., 138: 83. 1941.

(28) SALMON, T. N. The Effect on the Growth Rate of Thyroparathyroidectomy in Newborn Rats and of the Subsequent Administration of Thyroid, Parathyroid and Anterior Hypophysis. Endocrinology, 23: 446. 1938.

(29) SALTER, W. T. The Endocrine Function of Iodine. Harvard Univ. Press, Cambridge, Mass. 1940.

(30) SIEGERT, F. Affections of the Thyroid, in von Pfaunder, M., and Schlossmann, A., Diseases of Children, translated by S. W. Kelly. Philadelphia, J. B. Lippincott Co. Vol. 3, p. 534. 1912.

(31) SIMPSON, S. The Effect of Thyroidectomy on Growth in the Sheep and Goat as Indicated by Body Weight. Quart. Jour. Expt. Physiol., 14: 161. 1924.

(32) SIMPSON, S. Age as a Factor in the Effects Which Follow Thyroidectomy and Parathyroidectomy in the Sheep. Quart. Jour. Expt. Physiol., 6: 119. 1913.

(33) SNEDECOR, G. W. Statistical Methods. 3rd Ed., Iowa State College Press, Ames, Iowa. 1940.

(34) VAN LANDINGHAM, A. H., HENDERSON, H. O., AND WEAKLY, JR., C. E. Effect of Iodinated Casein (Protamone) on Milk and Butterfat Production and the Ascorbic Acid Content of the Milk. Meeting Amer. Chem. Soc. (Pittsburgh), Sept. 6–10, 1943. (See also JOUR. DAIRY SCI., 27: 385. 1944.)

VOLUME XXVII SEPTEMBER, 1944 NUMBER 9

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
Columbus, Ohio

MILK AND MILK PRODUCTS

Published in cooperation with
INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
R. C. HIBBEN, 1105 Barr Bldg., Washington, D. C., Exec. Sec.

INTERNATIONAL ASSOCIATION OF MILK DEALERS
R. E. LITTLE, 309 W. Jackson Blvd., Chicago, Illinois, Exec. Sec.

Editorial Committee

H. H. SOMMER, Madison, Wisconsin, A. D. S. A.
HAROLD PRATT, Philadelphia, Pennsylvania, I. A. I. C. M.
G. D. TURNBOW, Oakland, California, I. A. I. C. M.
W. D. DOTTERER, Chicago, Illinois, I. A. M. D.
A. J. POWERS, Brooklyn, New York, I. A. M. D.

CONTENTS

Bacteriology
Breeding
Butter
Cheese
Chemistry
Concentrated and dry milk; by-products
Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
Miscellaneous
Physiology

THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

PUBLICATIONS AND ABSTRACTORS

EDITORS

Dahle, C. D., Dahlberg, A. C., Horrall, B. E., Petersen, W. E., Tracy, P. H. and Weckel, K. G.

ABSTRACTORS

Archibald, J. G.
Babcock, C. J.
Bennett, F. W.
Berggren, Ruth E.
Burgwald, L. H.
Burkey, L. A.
Bushnell, L. D.
Call, A. O.
Cole, W. C.
Coulter, S. T.
Doan, F. J.
Dorsey, L. M.
Downs, P. A.
Ely, Fordyce
Erb, J. H.
Espe, D. L.
Frazier, W. C.
Fuller, S. A.
Glick, D. P.
Goss, E. F.
Henderson, J. L.
Huffman, C. F.
Irvine, O. R.
Josephson, D. V.
Lucas, P. S.
Lush, J. L.
Marquardt, J. C.
Mueller, W. S.
Price, W. V.
Pyenson, Harry
Reece, Ralph P.
Roberts, Henry E.
Thomsen, L. C.
Trout, G. M.
Waugh, R. K.
Webb, B. H.
Weckel, K. G.
Weiser, H. H.
White, G. C.
Yale, M. W.

JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics
Australian Journal of the Council for Scientific and Industrial Research

Biochemical Journal

Canadian Dairy and Ice Cream Journal
Canadian Journal of Public Health
Canadian Journal of Research
Certified Milk
Cornell Veterinarian

Dairy Industries
Dairy World

Endocrinology

Food in Canada
Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Medical Association
Journal of American Veterinary Medical Association
Journal of Animal Science
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
Journal of Genetics
Journal of Heredity
Journal of Industrial and Engineering Chemistry
Journal of Infectious Diseases
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology
Journal of Veterinary Research

Lancet
Le Lait

Milk Dealer
Milk Industry
Milk Plant Monthly

National Butter and Cheese Journal
New Zealand Journal of Science and Technology

Oil and Soap

Pacific Dairy Review
Proceedings of Society of Experimental Biology and Medicine

Refrigerating Engineering

Scientific Agriculture
Southern Dairy Products Journal

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BUTTER

263. Heat Resistant Coliform Organisms with Particular Reference to Butter. H. F. Long, T. I. Hedrick, and B. W. Hammer, Iowa Agr. Expt. Sta., Ames, Iowa. Jour. Milk Technol., *7*, No. 1: 20. Jan.–Feb., 1944.

Heat resistant coliform organisms were encountered in pasteurized milk, in cheese made from pasteurized milk, and in butter. Most of the studies were made on butter.

Of 116 cultures isolated from butter, 48 survived 61.7° C. for 30 minutes and 28 survived for 40 minutes. In some of the resistant cultures appreciable numbers of cells were still present after 50 minutes exposure at the above temperature. All resistant cultures were of the Escherichia types. No heat resistant Aerobacter cultures were encountered.

The authors state that "The general results suggest that Escherichia cultures isolated from pasteurized dairy products should be tested for heat resistance before assuming that pasteurization was inadequate or that contamination had occurred." L.H.B.

264. Starters and Cultures. M. W. Hales, Chr. Hansen's Lab., Milwaukee, Wis. Natl. Butter and Cheese Jour., *35*, No. 5: 24, May, 1944; and Natl. Butter and Cheese Jour., *35*, No. 6: 34, June, 1944.

This article is a review of what the nature and characteristics of starters and starter cultures should be. The author maintains that starters are selected for their ability to produce acid, flavor and aroma under commercial conditions and can be expected to do so if they are given careful uniform treatment from day to day and are renewed frequently. He warns that liquid cultures shipped by laboratories may contain small bubbles of carbon dioxide which are harmless. He strongly recommends the use of separate mother cultures instead of transfers of inoculum from bulk starter to bulk starter. W.V.P.

CHEMISTRY

265. The Solubility of Water in Butterfat. C. C. Thiel, Officer of Council. Jour. Council Sci. Ind. Res. (Austral.), *16*, No. 3: 139–141. Aug., 1943.

Determination of the solubility of water in butterfat over the temperature range of 40° to 95° C. showed that the solubility is 0.19 per cent at 40° C., 0.26 per cent at 60° C., 0.36 per cent at 80° C., and 0.47 per cent at 95° C.

Use of 8 per cent NaCl solution in place of water had no effect on the solubility, but mixed fatty acids from butterfat, when dissolved in fat to the

extent of two per cent, increased the solubility of water at 40° C. by 0.02 per cent. W.C.F.

266. The Determination of Moisture in Dried Butterfat. C. C. THIEL, Officer of Council. Jour. Council Sci. Ind. Res. (Austral.), *16*, No. 3: 135–138. Aug., 1943.

A comparison of different methods for the determination of moisture in butterfat showed that entrainment with nitrogen and the Fischer titration method gave similar results, but toluene distillation gave results 0.015 per cent lower. Heating methods gave consistent results equal to or only slightly lower than those obtained by the above methods, when conditions of heating were carefully standardized to yield maximum readings. Recommended heating methods were: (a) in a hot-air oven at 103° C. for three hours, (b) on a hot plate for 1½ to 2 minutes followed by 40 minutes in a vacuum oven at 100° C., or (c) on a hot plate at 160° C. for 3–4 minutes.

The Fischer titration method is rapid and uses a relatively small sample. W.C.F.

267. Tests for Formaldehyde in Milk. D. W. HORN, Bryn Mawr, Pa. Vol. 4, part 1, of the Pubs. of the Wagner Free Inst. Sci. Phila. 1944.

The author presents a study of the relative merits and limits of sensitivity of five different tests for the presence of formaldehyde in milk. He furthermore presents more specific directions for making these tests where specificity is now lacking. The tests investigated were: I, sulfuric acid—ferric sulfate test; II, hydrochloric acid—ferric chloride test; III, sulfuric acid—bromine test; IV, decolorized fuchsin test; and V, phenylhydrazine hydrochloride—sodium nitro prusside test.

The most sensitive test was found to be III; yet this test is not mentioned in the A.O.A.C. "Standard Methods." The least sensitive was V; yet this test is not vitiated by the presence of judiciously added nitrite as are the others. The major weaknesses of tests I, II and III are that they give negative results if the concentration of formaldehyde is too great. Methods of getting around this difficulty and the presence of nitrite and detecting very minute amounts of the preservative are discussed, as is also an efficient numbering and indexing system for studies of this general type involving ranges of concentration. F.J.D.

DISEASE

268. Too Little and Too Late. E. O. ANDERSON, Dept. of Dairy Indus., Storrs Agr. Expt. Sta., Storrs, Conn. Milk Plant Monthly, *33*, No. 4: 36–38, 40, 42, 50. 1944.

The author classifies the various types of mastitis and stresses the eco-

nomic and public health importance of mastitis. Some mastitis milk may become a real public health hazard if it is not properly cooled. Increasing attention is being given today to the mastitis problem. The presence of mastitis organisms in milk should be determined as early as possible. Such tests as (a) leucocyte count; (b) examination of incubated milk for streptococci occurring in chains; (c) colonies produced on special media; (d) reactions to the Hotis test; and (e) isolation of the causative organism and identification by fermentation reactions and serological grouping, may be used. These tests are used in the Connecticut Plan for the control of bovine mastitis.

The author lists seventeen suggestions for the prevention and spread of disease. In addition, he explains the use of chemotherapy in the control of infectious mastitis, which is briefly summarized under three conditions: treatment of milking cows, dry cows and those suffering from acute mastitis.

G.M.T.

269. Outbreak of Septic Sore Throat Due to Reconstituted Powdered Milk. Lt. Ralph F. Allen (MC), U.S.N., and Lt. Louis S. Baer (MC), U.S.N.R. Jour. Amer. Med. Assoc., *124*, No. 17: 1191–1193. April 22, 1944.

Here is reported what is believed to be the first epidemic of septic sore throat caused by milk from a contaminated "mechanical cow." Practically pure cultures of beta hemolytic streptococci were isolated from 155 throat cultures. Of two men assigned to work in the milk preparation room, one gave a history of having had a sore throat and a tender swollen gland in his neck two weeks before the outbreak. The epidemic subsided when the serving of milk was discontinued.

The authors make the following suggestions for the benefit of medical officers at stations or on ships using the "mechanical cow":

"1. Foremost in importance is careful instruction of enlisted personnel operating the machine and continued close supervision of their work.

"2. Sufficient men should be assigned to the job and an adequate number of 'mechanical cows' obtained to supply the needed quantity of milk without having to 'railroad it through.'

"3. A thorough breakdown of the mechanical cow is necessary daily with careful scrubbing of all parts with alkaline washing powder followed by steam sterilization for at least one minute.

"4. Weekly checks with a standard thermometer of both the pasteurizing and cooling temperatures is essential. A recording thermometer attached to the mixing tank of the mechanical cow is desirable.

"5. Particular attention should be paid to keeping milk cans clean along the seams and prompt retinning of those that rust.

"6. Careful screening and adequate ventilation are important and often overlooked items of sanitation.

"7. Drying racks for the milk cans of approved construction are easily made and should always be used. Too often cans are placed upside down on the deck.

"8. Bulkheads should be kept painted white to encourage cleanliness and expose dirt.

"9. Frequent checks on the health of all milk handlers is an important duty of the medical officer." D.P.G.

270. Cheese Carrying Typhoid in Alberta. ANONYMOUS. Canad. Dairy and Ice Cream Jour., *23*, No. 4: 40. 1944.

An outbreak of thirty-five cases of typhoid fever in southern Alberta was finally traced to cheese. Investigation indicates that the cheese became infected through the water supply. A. G. Campbell and J. Gibbard, Laboratory of Hygiene, Department of Pensions and Health, Ottawa, Canada, added *E. typhosa* to several experimental vats of milk which were manufactured by a commercial process into cheddar cheese. Under certain storage conditions they found viable *E. typhosa* after six months in all samples and for ten months or more in a majority of them. H.E.R.

FOOD VALUE OF DAIRY PRODUCTS

271. Influence of Incubation at 37° C. on Stability of Thiamin and Riboflavin in Cow's Milk. BARNETT SURE AND ZENAS W. FORD, Univ. of Ark., Fayetteville, Ark. Soc. Expt. Biol. and Med. Proc., *54*, No. 1: 83. Oct., 1943.

Pasteurized cow's milk was used throughout the experiment. It was found that incubating cow's milk for 22 hours at 37° C. caused 46% of the thiamin to be destroyed. Under similar treatment riboflavin was entirely stable. The critical temperature for thiamin destruction appeared to be between 31 and 37° C. The factors in the milk responsible for thiamin destruction were stable to 100° C. for 10 minutes. The minerals of cow's milk appeared to be responsible for about 25% of the thiamin destroyed. R.P.R.

272. Comparison of the Nutritive Value of Dextrose and Casein and of the Effects Produced on Their Utilization by Thiamine. CURT P. RICHTER AND KATHERINE K. RICE, Johns Hopkins Hosp., Baltimore, Md. Amer. Jour. Physiol., *141*, No. 3: 346. May, 1944.

Sixteen rats which had access to only one foodstuff, casein and water, survived on the average 33 days while 21 rats on dextrose and water sur-

vived 37 days. The rats on casein immediately became very inactive while the rats on dextrose became even more active than on a stock diet, at least for the first 20 days. The casein rats ate only approximately one-third as much food as the rats on dextrose.

When given access to thiamin hydrochloride, 14 rats on casein survived 55 days, while 12 rats on dextrose lived 73 days. The results of these experiments thus showed under the simplest conditions that thiamine plays an important part in the utilization of protein as well as of carbohydrate.

D.L.E.

273. **Food Plays "Ambassador" Role.** MRS. JULIA KIENE, Director, Westinghouse Home Economics Institute, East Pittsburgh, Pa. Certified Milk, *18*, No. 212: 7. Dec., 1943.

American food can be used as an "Ambassador" toward guaranteeing a lasting peace. The Army will require 15 per cent of the total supply of butter and 32 per cent of canned milk. American food will be needed in relief programs in newly liberated countries in order to prevent worldwide epidemics. Food habits, while difficult to change, will have to vary as the war progresses. Items stressed are; advertising to influence every American to produce, conserve, preserve fresh foods, share, eat the right food regardless of personal preferences, substitute plentiful for scarce foods, help keep food costs down, participate in community food projects, and to cooperate. H.G.L.

274. **Dairy Research and Human Nutrition. Parts I and II.** O. E. REED, U.S.D.A., Washington, D. C. Certified Milk, *18*, No. 212: 3, Dec., 1943; and Certified Milk, *19*, No. 213: 5, Jan., 1944.

See Abstract 99, JOUR. DAIRY SCI., **27**, No. 3: A47. 1944. H.G.L.

275. **The Biggest Bargain in Health.** MRS. JULIA KIENE, Director, Westinghouse Home Economics Institute, East Pittsburgh, Pa. Certified Milk, *19*, No. 214: 7. Feb., 1944.

The importance of milk as a food, together with the proper handling of milk in the home, is described. Suggestions are given on how to cook with milk to prevent excessive loss of vitamin B_2 and how to prevent curdling when cooking milk. Several recipes using milk are given. H.G.L.

276. **Nutrition and Health.** THOMAS PARRAN, Surgeon General, U. S. Public Health Service. Certified Milk, *19*, No. 214: 3. Feb., 1944.

In order to prevent malnutrition, it is advised that every citizen should have a down-to-earth working knowledge of modern nutrition and choose every day the food necessary for a balanced diet. H.G.L.

277. **Loss of Riboflavin in Milk Due to Sunlight.** OLAF E. STAMBERG AND D. R. THEOPHILUS, Univ. of Idaho, Moscow, Ida. Milk Dealer, *33*, No. 8: 32–42. May, 1944.

Since it is known that exposure to light destroys riboflavin, a series of experiments was conducted to determine the effects on riboflavin content of letting bottled milk stand on doorsteps. Mixed Holstein and Jersey milk, raw, pasteurized and homogenized, was bottled in plain and colored (brown) glass bottles and in paper bottles. Recognized tests for riboflavin content were used. All bottles were stored in the dark at 42° F. before being taken outside for direct sunlight exposure. Three tables of results are given showing percentage loss of different containers and under different conditions. Approximately one-half of riboflavin in milk in plain glass bottles is lost in 2 hours. Homogenized milk, due to greater opaqueness, showed slightly lower loss. Both brown bottles and paper bottles protected milk from loss of riboflavin in ratio of 10% to 80% in 6 hours as compared with plain glass. Protection for ordinary glass bottles of milk should be provided by the housewife to insure against loss of riboflavin due to exposure to direct sunlight. C.S.T.

HERD MANAGEMENT

278. **To Clean Milking Machines.** EVERT WALLENFELDT, Dept. of Dairy Indus., Univ. of Wis., Madison, Wis. Milk Plant Monthly, *33*, No. 4: 27–28. 1944.

If cleaning the milking machine is to be carried out consistently by all dairymen, the procedure must be simple. Emphasis is placed on rinsing the machine immediately after milking. Following the rinse, the machine should be taken apart enough so that brushes will reach all surfaces. Following brushing, parts should again be rinsed in lukewarm water and assembled. The teat-cup assembly should be hung on a solution rack and filled completely with 0.5 per cent lye solution. All metal parts should be dried when not in use. Previous to milking, two gallons of fresh clean 100 ppm. chlorine solution should be drawn through the machine. The machine should be inspected regularly and any faulty parts replaced. Emphasis is placed on the daily cleaning immediately after milking, if the machine is to be kept in good order. G.M.T.

279. **How to Sell Producers on the Value of Clipping Cows.** MELVIN A. MEYER. Milk Plant Monthly, *33*, No. 1: 31. Jan., 1944.

Clipped cows play an important role in clean milk production. The chief obstacle in the way of clipping cows is the clipper itself. These are frequently too expensive for the small dairyman to own, and a cheap hand clipper will not do the job. A solution to this problem confronting the small

dairyman would be for the dairy plant to own a few clippers which could be loaned to producers. A plant fieldman might be supplied with one clipper with which he could clip part of the cows in various communities in his milk shed as a demonstration of the value of clipping. Clipping of a cow's flank is no cure-all for unclean and unsatisfactory milk. Cleanliness yet remains a fundamental requirement in quality milk production. G.M.T.

280. Cold Weather Care of Milking Machine Rubber Parts. C. K. JOHNS, Div. of Bact. and Res., Sci. Serv. Dept. of Agr., Ottawa. Jour. Milk Technol., *7*, No. 1: 17. Jan.–Feb., 1944.

The use of a weak lye solution for maintaining the rubber parts of milking machines in a good sanitary condition has been definitely recognized by many investigators. However, it has one drawback in that the solution will freeze during cold weather.

In a study made to note the effect of a five-minute treatment of lye after a suction rinse with cold water, it was found that the lye solution was more effective than it had been previously realized.

First, a four-week study was made as follows: After a suction rinse with cold water, the teat cup assemblies of the units in use at the Central Experimental Farm were filled with lye solution, which had been kept in a cold storage room at near freezing temperature. After five minutes, this solution was drained out, and the teat cup assemblies remained in the cold room until next milking. During this four-week trial the assemblies were not dismantled nor brushed and appeared quite clean at end of the period.

The inner surfaces of the inflations and tubes were rinsed with sterile water and bacterial counts were made on the recovered rinse water, and on the milk of the first cow milked with each unit. This was repeated three times during the four-week period. The average count on the rinse waters were 470 per ml. and 2,600 per ml. for the milk samples. Then to note the effectiveness of this five-minute treatment with lye solution, when the teat cup assemblies were held between milkings at higher than freezing temperatures, the assemblies after treatment for the next seven weeks were held at temperatures between 70° and 80° F. As in the previous trial, they were not dismantled nor brushed during this period. Bacterial counts were made four times during this period in the same manner as previously. Results for the rinse waters averaged 550 per ml. and for the milk samples 3,500 per ml.

After these preliminary trials it was decided to try this treatment for a year with storage of teat cup assemblies at room temperature. During this time, however, the teat cup assemblies were taken apart every week or ten days as was the customary practice.

At these periodical inspections there was no evidence of fat or milk solids being present on the parts. There was a slight deposit of calcium

phosphate in some of the inflations after they had been in use several months. The monthly logarithmic average bacterial counts of the mixed night's and morning's milk (raw) taken from the pasteurizing vat for the year 1941–42 ranged from 5,768 to 21,530, while for 1942–43 they ranged from 4,477 to 20,000. L.H.B.

ICE CREAM

281. The Present and Future Role of the Ice Cream Industry in Frozen Foods. F. L. THOMPSEN, Bur. Agr. Economics, U.S.D.A., Washington, D. C. Ice Cream Trade Jour., *40*, No. 6: 10. June, 1944.

The author reports on a comprehensive survey of the present and potential future developments of the frozen food business in the ice cream industry. Of the total civilian pack of frozen foods in 1942, the ice cream industry distributed 5.6 per cent. Ten per cent of all retail ice cream outlets sold frozen foods, and 67 per cent of these utilized regular ice cream cabinets for holding the products. Studies indicated that ice cream manufacturers are in a particularly favorable position to embrace this additional activity, either by acting as distributors of foods frozen by another organization or by actually utilizing excess freezing space for freezing the foods themselves. Methods of operation are discussed and equipment of various types are described and evaluated. The costs will vary considerably with various factors, but estimates are mentioned which will give the ice cream manufacturer some idea of the potentialities of the enterprise. F.J.D.

282. Research-Use of Banana in Dehydrated Form. B. I. MASUROVSKY, Res. Editor. Ice Cream Trade Jour., *40*, No. 6: 38. June, 1944. F.J.D.

283. "Velva Fruit" Research. B. I. MASUROVSKY, Res. Editor. Ice Cream Trade Jour., *40*, No. 6: 28. June, 1944. F.J.D.

284. Outlook on Vanilla. RAY C. SCHLOTTERER, Vanilla Bean Assoc. of Amer., Inc. Ice Cream Field, *43*, No. 6: 18. 1944.

The author, in discussing the Bourbon vanilla situation, points out that normally about 60 per cent of our vanilla beans come from Madagascar, but that in the past three years only two shipments have been made direct from there. Beans seized by the British from French vessels attempting to evade the blockade also have been received on five occasions during that period. One shipment of over 100 tons is expected to arrive in the United States this year, it is claimed. The French are creating, and will continue to maintain, a substantial supply of vanilla in Madagascar for the mother country, the author states.

It is estimated that this year's crop of Mexican vanilla will amount to about 275,000 pounds, and the author states that the quality is reported good. Due to drouths in Mexico this year, next year's crop is expected to be low. The author estimates it will not be more than 175,000 pounds.

Tahiti vanilla has been arriving at fairly regular intervals, and it is stated that the price there has been stabilized. The price situation as a whole, however, is moving upward due primarily to uncertainty over Bourbon arrivals. It is pointed out that, if prices advance too far, vanillin will be used as a substitute for vanilla, which will eventually cause prices to decrease. Prospects for a decrease in price in the near future are very remote, it is stated. W.C.C.

285. Tests for Quality in Egg Pulp. 1. A Preliminary Note on the Application of the Reductase Test Using Resazurin as the Indicator. W. J. SCOTT AND J. M. GILLESPIE (Div. of Food Preservation and Transport, Austral.). Jour. Council Sci. Ind. Res. (Austral.), *16*, No. 1: 15–17. Feb., 1943.

The time of reduction of resazurin to a pink color afforded a reliable prediction of the bacterial content of the pulp, since results correlated with the plate count. The type of flora influenced the reduction time, but the organisms were mostly *Pseudomonas*. Two milliliters of 0.005 per cent resazurin were mixed with 10 ml. of egg pulp and incubation was at 30° C.

Data given indicated a reduction time of about 5 hours when a million bacteria per ml. were present and about 2 hours with a billion. W.C.F.

286. Report of Committee on Frozen Desserts. F. W. FABIAN, Mich. State Col., East Lansing, Mich. Jour. Milk Technol., *7*, No. 1: 31. Jan.–Feb., 1944.

Although these are especially trying times, inspectors and dairymen alike should be made to appreciate the necessity of keeping dairy products sanitary. The essentials for which there should be no relaxation of vigilance are health of cows and employees; cleanliness of equipment, utensils, cows, and employees; and effective pasteurization.

It is suggested that the dairy inspector concentrate on the essentials, and let the rest go for the duration.

Reports by O. A. Ghiggoile, of California; John M. Scott, of Florida; and Andrew J. Krog, of New Jersey, are given. L.H.B.

287. Specific Gravity and Overrun in Frozen Ice Cream. P. S. LUCAS AND ROBERT E. STOUT, Dept. of Dairy Husb., Mich. Agr. Expt. Sta., Lansing, Mich. Canad. Dairy and Ice Cream Jour., *23*, No. 5: 35. 1944.

A new test to determine specific gravity and overrun in ice cream.

Present formulas depend on knowing the weight or volume of mix used in making a known amount of ice cream. Such formulas are obviously impracticable for inspectors. The new method proposed permits overrun determination in a sample of frozen ice cream.

A 130-gram portion of sample was melted and poured into a tared volumetric flask. Ten grams of normal amyl alcohol sp. grav. 0.817 were added to release air from the air cells. Water was then added to make the volume to 250 cc. Flask and contents were then weighed and the weights of the contents calculated.

To illustrate:

Weight of flask and contents	335.75 grams
Weight of flask	76.75 "
Weight of contents	259.00 "
Weight of water (259–(130–10))	119.00 "
Volume water and alcohol (119.00–12.24)	131.24 ml.
Volume occupied by sample (250–131.24)	118.76 "
Specific gravity of mix (130 ÷ 118.76)	1.0946

Knowing the specific gravity of the mix, it is easy to calculate the per cent of overrun.

Twenty tests were made with the above method on samples of known overrun with very satisfactory results. H.E.R.

288. Stabilizing Agents in Mix Building. KENNETH M. RENNER, Dept. Dairy Manufactures, Tex. Technol. Col., Lubbock, Tex. South. Dairy Prod. Jour., *35*, No. 4: 22. April, 1944.

At the Texas Technological College corn flour affected the flavor of ice cream less than did soybean flour or wheat flour. One per cent corn flour had an effect equal to two per cent wheat flour or three per cent milk solids not fat when added to a mix containing 8.8 per cent milk solids not fat. Two per cent corn flour caused a slick corn-starchy consistency. The use of additional vanilla flavor minimized the flour flavor. A slightly greater whipping ability resulted from the use of corn flour.

The use of vegetable flours results in an ice cream which is acceptable, but it is inferior to a high quality ice cream where the solids are derived entirely from good milk products. F.W.B.

289. Chemical Flavor in Ices and Sherbets. B. I. MASUROVSKY, Res. Editor. Ice Cream Trade Jour., *40*, No. 5, 50. May, 1944.

F.J.D.

MILK

290. A Comparison of the Roll-Tube and Standard Plate Methods of Making Bacterial Counts of Milk. C. C. PROUTY, H. A. BENDIXEN, AND S. P. SWENSON, Div. of Dairy Husb. and Div. of Agron., Wash. Agr. Expt. Sta., Pullman, Wash. Jour. Milk Technol., *7*, No. 1: 5. Jan.–Feb., 1944.

The use in Europe of a roll-tube technique for the quantitative and qualitative analysis of milk and other products was observed by one of the authors. Since this technique appeared to possess certain advantages over the standard plate method, a study was undertaken for the purpose of comparing the results from the two methods.

A description illustrated by pictures of the roll-tube method is given.

Two series of comparisons between the roll-tube method and the standard plate method are given. One series was of 6 to 20 replicate tests made by each method on 14 samples of milk, while the other series was of duplicate tests made by each method on 43 samples of milk.

The counts obtained by the roll-tube method generally were lower than those obtained by the standard plate method. This was especially true in high colony count samples where the number of colonies approached 300, and probably was due to the fact that the surface area of the agar in the tubes was smaller than that of the plates, thus causing increased crowding of the colonies in the tubes.

The advantages given for the roll-tube method are that the tubes are subject to less breakage and require less storage space. They can be filled with the required amount of media, sterilized, and kept on hand for instant use. They can be readily mailed for use in the field where limited laboratory facilities are available. The tubes require only 7 ml. of agar instead of the 10–15 ml. needed for standard plates; also, less incubator space is required.

Disadvantages are—a special motor-driven apparatus is required for rotating tubes; more difficult to count colonies on tubes than on plates, especially when number of colonies approach 300; need a firmer agar (2.0%) for tube method; possibility of spreading colonies greater in tubes than in plates; tubes must be incubated with their bottom ends slightly sloping downward. L.H.B.

291. Production of 18% Cream with Best Physical Properties. A. C. DAHLBERG, Cornell Univ., Ithaca, N. Y. Milk Dealer, *33*, No. 7: 36–40. April, 1944.

A light cream, in addition to fine flavor, needs: (1) maximum viscosity, (2) no cream plug, (3) no skim milk layer, (4) no oiling off in coffee, (5) no feathering in coffee, and (6) maximum ability to color coffee. To obtain these often incompatible properties in 18% cream, the author suggests (1)

avoidance of churning or excessive agitation during transportation, (2) proper separation of high-test milk if available, (3) proper pasteurization, (4) slow cooling to increase viscosity, (5) heat treatment to increase viscosity, and (6) proper homogenization. With increased demands for milk fat in other dairy products an homogenized milk-cream mixture of 10% fat correctly labeled and sold may be a practical answer to the demand for a product more than fluid milk for use in coffee or on cereals. C.S.T.

292. Dairy Equipment Must Be Free from Microbial Contamination. W. L. MALLMAN, Dept. of Bact., Mich. State Col., East Lansing, Mich. Milk Plant Monthly, *33*, No. 2: 23–25. Feb., 1944.

In quality milk production the equipment must be physically clean and free from objectionable micro-organisms. Sanitation of equipment includes both the removal and destruction of the bacteria which may be present. In destroying micro-organisms the killing agent must either enter the organism or must come into intimate contact with its cell wall. Hence if the organism is completely covered with organic or inorganic soil, such as milk proteins, milk stone or water stone, it is practically impossible to reach the cell with any acceptable chemical or physical sanitizer. Particularly is this true in the case of milk containing thermoduric bacteria. Proper cleaning removes most of the bacteria from the utensils. Sterilizing agents are used primarily as a safety measure and assure further reduction in bacterial populations. The selection of a sterilizing agent is important, and whether heat or chemical depends largely upon the available equipment and the personnel. Heat is the most effective method of destroying microbial life. It has the advantage of penetrating through organic and inorganic matter and destroying the bacteria. However, it has the disadvantage that sufficient temperatures are not always employed, hence the use of heat may give the producer a false sense of security, particularly when thermoduric organisms are present. Chemical sterilization of dairy equipment by the use of chlorine in one of several forms should be recommended where heat is inadequate or improperly used. Chlorine has the quality of acting rapidly even in the absence of heat. Its chief disadvantage is its lack of penetrability. Like heat, chlorine is also dissipated in use. Time of exposure to chlorine is very important. When chloramine compounds are used, the exposure period should be longer to compensate for its slower action. The importance of sanitation in dairy equipment cannot be over-emphasized. G.M.T.

293. Milk Flavor from the Producer's Standpoint. J. L. HENDERSON, Dairy Indus., Col. of Agr., Univ. of Calif., Berkeley, Calif. Milk Plant Monthly, *33*, No. 5: 40, 41. 1944.

By following a few basic principles, a fine-flavored milk may be produced consistently. Many feed flavors are under the control of the pro-

ducer. Feed flavor is perhaps the most common flavor defect which may be controlled by the producer. To eliminate feed flavors from the milk, feed-flavor-producing substances should not be fed during a five-hour period before milking. Salty tasting milk should be eliminated from the general supply. The development of rancidity may be prevented by mixing potentially rancid milk with normal milk in the ratio of one to four or five just before or after cooling. The major control problem of oxidized flavor lies largely with the distributor, although the producer is not free from all responsibilities. The responsibility of the producer with respect to oxidized flavor development appears to be (1) to feed and maintain the milking herd in the best possible condition, and (2) to keep the milk out of copper contact. G.M.T.

294. Maintaining Consumer Confidence in Milk Supplies. KENNETH M. RENNER, Head, Dept. Dairy Manufactures, Tex. Technol. Col., Lubbock, Tex. Milk Plant Monthly, *33*, No. 1: 36–40. Jan., 1944.

With a marked increase in consumption of milk, dairymen have been confronted with the problem of supplies. With high prices, increased labor costs and acute shortage of labor, many dairymen have considered quitting business, or securing a supply of milk not complying with the established standards. A shortage of Grade A milk has made it necessary to use milk other than Grade A. In sections where consumers have become attached to the Grade A label, it is necessary to inform them why the label was taken off the milk. People drink milk because they like it. Flavor is emphasized. Good milk is clean milk, free from sediment, produced from healthy cows, having no off-flavors and a relatively low bacteria count. All milk for resale to the consuming public should be pasteurized. Volunteer consumer rationing will assist materially in keeping the confidence of the consumer. This may be accomplished through a questionnaire to the consumer explaining the milk situation. The author lists 10 points for maintaining the milk supply. Cooperation among the various groups involved will aid materially in solving the problems of the shortage of milk supply and maintaining consumer confidence in milk. G.M.T.

295. Planning for Future Operations in the Market Milk Industry. P. H. TRACY, Univ. of Ill., Urbana, Ill. Milk Dealer, *33*, No. 8: 112–115. May, 1944.

The market milk plant of the future must reduce inefficiency in plant operation in order to survive in the post-war period of over-expanded markets, increased milk sales through stores, increased bottling by chain stores, centralization of pasteurization units and increased integration. The author lists thirty conditions contributing to greater efficiency in milk plant opera-

tion in order to reduce costs and enable the post-war milk plant to survive. The sales advantages of fresh bottled milk as compared with powdered, evaporated or reconstituted milk in the post-war period are: (1) usually superior in flavor, (2) fresher, and (3) more convenient for the housewife to use. The operator of the milk plant of the future must have good business judgment, understand labor, have a technical knowledge of business and hire a man "capable of adapting his ideas to the operation of his business." C.S.T.

296. Testing Pasteurized Milk. A. L. PROVEN, Harper Adams Agr. Col., Newport, Salop, AND A. ROWLANDS, Midland Agr. Col., Sutton Bornington, Loughborough. Irish Agr. and Creamery Rev., *13*, No. 123: (new series) 11–15. March, 1944.

The need for bacteriological examination of pasteurized milk is primarily to determine recontamination after pasteurization and to estimate keeping quality in the hands of the consumer. Therefore, simple tests other than colony and coliform counts should be carried out to ascertain the type of organisms which are carried over or recontaminate the milk, and which influence the keeping quality of bottled pasteurized milk held at normal household temperatures. To assess their effect under wartime conditions of processing milk, the authors took a survey of over 40 plants in the Midlands and found that, both bacteriologically and in keeping qualities, bottled milk had deteriorated greatly. The possibility of utilizing some simple test to supplement complete laboratory tests was therefore studied.

Results of the methylene blue test on raw and pasteurized milk at 15.5° C. (Hiscox *et al.*, Jour. Dairy Res., 4: 105) showed that in relation to keeping quality and to degree of recontamination (Rowlands and Proven Proceeding, Soc. Agr. Bact., Eng., 29) a reduction of 1½ days or less is indicative of spoilage before use by the consumer. In the experiments conducted, pasteurized commercial samples were aged 24 hours before testing. Tables are given showing results—6 hours or less after preliminary aging of 24 hours. Similar tests after aging 24 hours were conducted with the resazurin test and results tabulated. Incubation at both 15.5° C. and 18° C. for 24 hours were used and tested at 37° C. with results deemed indicative of degree of souring and deterioration occurring during aging. Several hundred samples of different milks were tested by these two modified methods and tables published. The procedure for the modified resazurin test for control of pasteurized bottled milk, including sampling, incubation, testing and interpretation of results, is given. The use of this test is summarized as follows: (1) test not applicable to freshly pasteurized milk; (2) preliminary incubation for 24 hours at 15.5° C. or 18° C. is necessary to be indicative of recontamination and keeping quality; (3) the resazurin test made at 37° C. after incubation is suitable for plant control check in

judging efficiency of cleaning and sterilization of processing plant; and (4) the results of dye reduction tests are not related to the colony count at 37° C. and do not indicate compliance with the official standard. Fourteen references are cited. C.S.T.

297. Fieldman's Share in Producing Quality Milk—IV. Fieldman's Care of Can Washer. VERN P. MELHUISH. Milk Plant Monthly, *33*, No. 1: 27–31. Jan., 1944.

In addition to receiving and discharging cans, the can washer must also execute five operations, namely: (1) rinse all milk solids from each can; (2) wash each can; (3) rinse each can with hot water at minimum temperature of 180° F.; (4) practically sterilize each can with sterile rinse water and live steam; and (5) remove steam vapors and complete the drying of each can with air. Since the fieldman must explain to the producer any shortcomings of can washing, it behooves the fieldman to assume more responsibility in the receiving room operations, particularly the can washer at the receiving plant. Thus the fieldman must be thoroughly familiar with the particular can washer at the plant and know what kind of trouble to look for and where to look for it. This should not lessen the general responsibility of can washing supervision. By using a "test can," from which a 10″ strip has been removed from each side, the operator may observe the operation of each jet over which it passes. The author lists ten steps in procedure for testing the can washer, and furnishes a list of items which should be checked weekly if the can washer is to be kept in good operation. G.M.T.

298. Fieldman's Share in Producing Quality Milk—V. Fieldman Must Sell Producers on Quality Production. W. L. SLATTER, Instr. of Dairy Technol., Ohio State Univ., Columbus, Ohio. Milk Plant Monthly, *33*, No. 2: 22–23. Feb., 1944.

Emphasis is placed on the fact that the dairyman himself is the most important factor in the production of high quality milk. The fieldman can do much in attaining high quality milk production by emphasizing to the producer that quality milk production is not difficult, but rather doing a few necessary things at the right time. The necessary equipment and rules for production of high quality milk are listed and discussed. G.M.T.

299. Fieldman's Share in Producing Quality Milk—VI. Practical Aspects of Chlorine and Heat Sterilization of Dairy Equipment. M. E. MCDONALD, Bur. of Dairy Serv., Calif. Dept. Agr., Sacramento, Calif. Milk Plant Monthly, *33*, No. 3: 30–32. March, 1944.

The author compares chlorine and heat as sterilizing agents for dairy equipment. A diagram of an oil-burning hot water and steam generator

is included. Procedures and precautions in using sterilizing agents under various conditions are given. Cleanliness, sterilization, and drying are essential requirements in preparing dairy equipment for use. G.M.T.

300. Fieldman's Share in Producing Quality Milk—VII. Keeping the Milker Clean. GEORGE H. HOPSON, DeLaval Separator Co. Milk Plant Monthly, *33*, No. 4: 26–27. 1944.

The author emphasizes that the milking machine should be kept clean and never allowed to become dirty. Facilities or conditions for keeping equipment clean are especially important, and contain such items as (a) desire to keep milker clean; (b) ample quantities of cold water; (c) warm and hot water; (d) brushes; (e) lye and chlorine; and (f) dairy washing powder.

Keeping the milking machine clean involves immediate rinsing, dismantling, brushing and sterilization. Immediate rinsing is probably the most important. Sterilization may be effected through hot water at 180° F. or above, or if not available, a chlorine rinse containing 250 ppm. of chlorine may be used. G.M.T.

301. Fieldman's Share in Producing Quality Milk—VIII. RUSSELL R. PALMER, Chief Milk Insp., Dept. of Health, Detroit, Mich. Milk Plant Monthly, *33*, No. 5: 52–53. 1944.

Sediment in milk consists of any material or substance that was not in the milk as it came from the udder of a healthy cow. The milk may be sampled for sediment by one of several ways, namely, by drawing the milk off the bottom of the can, collecting the last of the milk as poured from the can, or taking a sample of milk from a well-stirred can. Each method is designed and employed to determine the physical cleanliness of the milk. Sediment will not increase in amount in the milk, but the bacteria associated with it may increase in numbers. The presence of sediment in milk indicates poor sanitation, and poor methods of production and handling of milk. Inasmuch as physically clean milk improperly cooled may show a high bacteria count and, conversely, high sediment milk adequately cooled may show a low bacteria count, there is no consistent relationship between the sediment test and the bacterial content of the milk. Either high sediment or high bacteria count represent unsanitary conditions and demand immediate investigation and correction. As such, both tests are definitely useful, but not definitely related to one another in results. G.M.T.

302. Fieldman's Share in Producing Quality Milk. IX. Filtration of Milk on the Farm. BROR E. ANDERSON AND JAMES A. ANDERSON, Johnson and Johnson, Chicago. Milk Plant Monthly, *33*, No. 6: 26–30. 1944.

Use of a reliable filter disc will not only remove dirt from the milk, but

a study of the used disc will indicate the needed precautions in reducing the amount of dirt falling into the milk. Important reasons for filtering milk on the farm even though the milk may be subsequently filtered at the milk plant are (a) to remove extraneous matter from the milk as soon as possible, (b) to serve as convenient farm sediment check to note the type of sediment in milk, (c) to encourage production of milk with decreased amount of sediment in it, (d) to aid in detecting mastitis, and (e) to lighten the burden on milk plant filters. A good milk filter should be fast, efficient, single-service with the fibers bonded together so they will not tear apart under pressure. Filtering of milk should be done promptly after milking while the milk is still warm. Bumping of the strainer to hasten filtering should not be tolerated. Filtration is an aid in clean milk production rather than in "cleaning" milk. G.M.T.

303. **Preventing Defects in Bottled Milk and Cream.** E. L. FOUTS, Agr. Expt. Sta., Gainesville, Fla. South. Dairy Prod. Jour., *35*, No. 4: 85. April, 1944.

Wartime conditions have tended to increase defects in bottled milk and cream because: (1) New supplies of milk have been necessary to supply demand; (2) It has been necessary to depend largely on inexperienced employees; (3) There is a shortage of equipment and much machinery is badly worn; (4) Limitations on transportation have necessitated less frequent delivery.

Two of the most important off flavors are oxidized flavor and rancidity. The first is usually caused by exposure of the product to unplated copper in equipment or to direct sunlight. Every effort should be made to avoid this exposure. The latter may be caused by enzymes present in the milk when it is produced or by microorganisms. This flavor defect is particularly troublesome in homogenized milk. Pasteurization before the flavor develops will usually prevent this defect.

Bacteriological problems generally are the result of careless methods in handling the milk, insufficient cooling, and failure to properly sterilize containers and equipment. Coliform organisms may indicate fecal contamination. The remedy is the prevention of the contamination by clean methods of production, the use of sufficient refrigeration, efficient pasteurization and sterilization of equipment. Poor keeping quality is associated with the same conditions which cause bacteriological problems.

The most common other defects in cream are cream plug, oiling off, feathering, and the occurrence of a skimmilk layer. Cream plug is characterized by a tough leathery mass at the top of the bottle caused by partial churning. This defect may be prevented by avoiding excessive or prolonged agitation, especially when warm, and avoiding freezing. Oiling off is closely associated with cream plug and remedy is the same.

Feathering is a heat coagulation of the protein of the cream. This condition usually can be remedied by the addition of two to six ounces of sodium citrate to 1,000 pounds of cream.

Two-stage homogenization at 1,000 plus 500 pounds pressure after pasteurization will reduce or eliminate the occurrence of a skimmilk layer at the bottom of the bottle. Whether or not the cream is homogenized, the body of the cream will be improved by an aging period of 24 hours at 35 to 40 degrees Fahrenheit before bottling. F.W.B.

304. The Value of the Milk Serum Agglutination Test in Safeguarding Raw Milk Supplies. H. E. BREMER, Vt. Dept. of Agr., Montpelier, Vt. Jour. Milk Technol., *7*, No. 1: 26. Jan.–Feb., 1944.

In view of the fact that the use of raw milk from Bang infected cows presents an important health control measure, some quick test is desirable. The milk serum test is used for this purpose as a screen test. Samples are taken from each can of milk produced for sale as raw milk. A positive or suspicious test demands that either the herd be officially blood tested and reactors removed or that the milk be pasteurized.

A comparison was made of the milk serum and blood serum agglutination tests for Bang's Disease on 472 cows in 31 Vermont herds. In the majority of instances less than 60 days elapsed between the milk serum tests and the blood serum tests, but in a few instances they were more than 90 days apart.

The results showed that 71.8% (339 cows) of the milk serum and blood serum tests checked. 10.8% (51 cows) of the negative tests on milk were suspicious on blood. 12.1% (57 cows) of the negative tests on milk were reactors on blood. 4.4% (21 cows) of the suspicious tests on milk were reactors on blood. 0.9% (4 cows) of the reacting tests on milk were suspicious on blood.

The milk serum test, if used as a basis for determining reactors, would undoubtedly overlook some reactors. Almost invariably the blood test will give a stronger reaction than will the milk. L.H.B.

305. The Flavour Quality of Milk. W. H. SPROULE, Ontario Agricultural College, Guelph. Canad. Dairy and Ice Cream Jour., *23*, No. 4: 46. 1944.

Emphasis should be placed on the desirable flavors of milk instead of stressing the bad flavors. Experiments by Roadhouse and Koestler, California, show that the chlorides and lactose of milk have more to do with taste than any of the other constituents of milk. Superb flavor usually accompanies a high lactose and a low chloride content. Percentages of chlorides, lactose and fat change during lactation, and cow freshening dates should be staggered wisely.

Feed flavors may be overcome by withholding feed from the cows before milking. Milk readily absorbs flavors and should be kept in a clean, well-ventilated place. Other causes of bad flavor are bacteria, physiological disturbances in the cow and chemical activity induced by sunlight, metallic salts and enzymes. H.E.R.

306. **Delivery Problems Today and Tomorrow.** J. N. BAUMAN, Vice-Pres. and Sales Mgr., The White Motor Co., Cleveland, Ohio. South. Dairy Prod. Jour., *35*, No. 4: 56. April, 1944.

At the beginning of the war there were some 4,500,000 motor trucks in service in this country. Conservative authorities have estimated that some 300,000 vehicles are disappearing from the highways annually. Since the number of new trucks built does not exceed 25,000 annually and they are allotted on highest priority standing, there are virtually no replacements available to private operators.

The first essential in maintenance is proper lubrication to avoid wear. Adjustments or replacement of worn parts at the proper time must be made to avoid damage to other parts. A maintenance manual should be available to the mechanic so that he may properly make adjustments and repairs.

After maintenance the second most important phase of truck care is the availability of parts when and where needed. The use of a parts book to identify the parts needed facilitates the filling of orders. The trucks for which parts are ordered should also be identified by model and serial number. The driver plays a very important part in the life of a truck. He should report danger signals to the maintenance department promptly. Full use of maintenance information from the truck manufacturer is important. Delivery service must, of necessity, be restricted as much as possible without extreme effect upon sales.

War-learned lessons on economical operations should be observed in peacetime since distributing cost will play a large part in the success of an enterprise following the war. F.W.B.

307. **Influence of Homogenization on the Properties of Milk and Cream.** J. L. HENDERSON, Univ. of Calif., Davis, Calif. Milk Dealer, *33*, No. 8: 30, 76, 78. May, 1944.

With the exception of the manufacture of butter, every branch of the dairy industry now uses homogenization. This process increases the number of fat globules, causes adsorption of protein from the milk plasma, and increases surface at the fat-liquid interface. It influences milk and cream by producing non-creaming properties; increases viscosity; and induces rancidity, oxidized and "sunshine" flavors more readily than in unhomogenized milk or cream. The claim that homogenized milk or cream tastes richer was not substantiated in this experiment. Homogenization also reduced curd tension irrespective of the original curd hardness, and possibly

increases digestibility. It produces a whiter color due to increase in the surface of the fat reflecting light and decreases protein stability. Homogenized milk will coagulate quicker than unhomogenized. The use of returned homogenized milk in ice cream and chocolate milk is probably the best means of utilization.

Directions for Babcock, U. S. Public Health Standard and Farrall index tests for homogenized milk are given. C.S.T.

PHYSIOLOGY

308. Initiation and Maintenance of Lactation in Dairy Heifers by Hormone Administration. RALPH P. REECE, N. J. Agr. Expt. Sta., New Brunswick, N. J. Soc. Expt. Biol. and Med. Proc., *52*, No. 2: 145. Feb., 1943.

A 30-month-old Holstein-Friesian heifer received a total of 163 mg. of diethylstilbestrol dipropionate and 60 mg. of testosterone propionate in 11 weeks. During the injection period there was a gradual increase in udder and teat size but at no time was the udder filled with secretion. The peak daily milk production was 27.5 lb. and in 7 months the heifer produced 4517.1 lb. A 33-month-old Jersey heifer received a total of 273 mg. of diethylstilbestrol dipropionate in 14 weeks. At first there was a slight increase in udder and teat size and then the udder appeared to become slightly filled with secretion. At the end of the injection period the udder had increased considerably in size but it was not distended with secretion. The heifer attained a peak daily production of 33.7 lb. three months after the last injection. R.P.R.

309. Differentiation between *Brucella melitensis* and *Brucella abortus* by Precipitin Reaction. P. MORALES OTERO AND A. POMALES LEBRÓN, School of Tropical Medicine, San Juan, Puerto Rico. Soc. Expt. Biol. and Med. Proc., *52*, No. 3: 197. March, 1943.

The precipitin reaction was used in an attempt to develop a simple and rapid method by which strains of *Brucella melitensis* could be differentiated from those of *Brucella abortus*. *B. abortus* and *B. suis* could not be differentiated; yet they could both be differentiated from *B. melitensis*. Three melitensis strains failed to precipitate in 30 minutes when tested against *abortus* and *suis* sera, but one strain did. Attention is called to the importance of using proper strains, typical in all respects, for immunization. R.P.R.

310. Secretion of Radio-active Sodium in Human Milk. W. T. POMMERENKE AND P. F. HAHN, Univ. of Rochester, Rochester, N. Y. Soc. Expt. Biol. and Med. Proc., *52*, No. 3: 223. March, 1943.

Four normal young women served as subjects and at the beginning of

each experiment the experimental breast was emptied with an electric pump. Each subject received a single dose of about 65 mg. of radio-active sodium, as the chloride, in orange juice. Radio-active sodium was recovered in milk within 20 minutes after its ingestion and its peak concentration was reached in about 2 hours. Subsequent secretion slowly tapered off, some of the sodium being recovered after 96 hours. R.P.R.

311. Lactation Activity, Chemical Composition and *in vitro* Metabolism of Rat Mammary Tissue. MAX KLEIBER, ARTHUR H. SMITH, AND PHILLIP LEVY, Univ. of Calif., Berkeley, Calif. Soc. Expt. Biol. and Med. Proc., *53*, No. 2: 94. June, 1943.

Mammary tissue of rats of the Long-Evans strain at the end of pregnancy and at the height of lactation was analyzed for dry matter and nitrogen content, and the metabolic rate of the tissue was measured *in vitro*. The water content of the mammary tissue and the protein content of the mammary dry matter increased during lactation. Lactation did not alter the metabolic rate per unit of fresh tissue; however, it increased the metabolic rate per unit of dry matter and decreased the metabolic rate per unit of nitrogen in the tissue. Additional experiments indicated a positive correlation between the intensity of lactation and the magnitude of the effect that lactation had on the composition and metabolic rate of mammary tissue. R.P.R.

312. Effect of environmental Stilbestrol in Shortening Prolonged Gestation in the Lactating Rat. CHARLES K. WEICHERT, Univ. of Cincinnati, Cincinnati, Ohio. Soc. Expt. Biol. and Med. Proc., *53*, No. 2: 203. June, 1943.

One tenth to 0.5 mg. of diethylstilbestrol scattered about the cages of inseminated lactating rats suckling 9 young brought about implantation at the normal time, day 6, rather than around the 16th day as in untreated controls. The importance of isolating experimental animals that are being treated with diethylstilbestrol was pointed out. R.P.R.

313. Occurrence of Premature Ovulation in the Domestic Fowl Following Administration of Progesterone. RICHARD M. FRAPS AND ABRAHAM DURY, Bur. of Anim. Indus. Soc. Expt. Biol. and Med. Proc., *52*, No. 4: 346. 1943.

Crystalline progesterone was shown to be highly effective in the domestic fowl when administered under certain conditions in causing premature ovulation of normally developing ovarian follicles. Route of injection, time from injection to expected normal ovulation, injection level, and place of the follicle in the clutch sequence influenced results. First follicles of clutch

sequences were ovulated at least 6 hours prematurely in 90 to 95% of hens injected with 0.5 and 1.0 mg. of progesterone intravenously, or 1.0 to 10.0 mg. subcutaneously. Follicles other than the first of clutch sequences were ovulated by at least 7 hours prematurely in 70 to 75% of the hens injected subcutaneously at dosage levels of 0.5 to 5.0 mg. progesterone. Under identical conditions intravenous injection of progesterone was relatively ineffective. R.P.R.

314. Pregnancy Maintenance in Hypophysectomized-Oöphorectomized Rats Injected with Estrone and Progesterone. WM. R. LYONS, Univ. of Calif., Barkeley, Calif. Soc. Expt. Biol. and Med. Proc., *54*, No. 1: 65. Oct., 1943.

Rats of the Long-Evans strain were caged with males and those showing vaginal sperm were hypophysectomized and spayed on days 7 or 8 of pregnancy. Hormonal injections were begun immediately after operations and continued until the day before autopsy. It was found that one microgram of estrone and 3 or 4 mg. of progesterone substituted for the ovaries and pituitary during the period of pregnancy between days 7 and 11 and for the ovaries alone from day 11 until term. It was concluded that the pituitary is only necessary during the first half of pregnancy in the rat and then probably because of its gonadotropic triad, FSH, ICSH, and lactogenic hormone. R.P.R.

315. Influence of Fat Mobilization on Acetone Body Production. I. ARTHUR MIRSKY, ISABELLE GRAYMAN, AND NORTON NELSON, Univ. of Cincinnati, Cincinnati, Ohio. Soc. Expt. Biol. and Med. Proc., *51*, No. 3: 363. Dec., 1942.

Two groups of ducks were fasted for 3 days and then one group was injected subcutaneously with 4 mg. of diethylstilbestrol. Every third day thereafter for 15 days blood samples were drawn for the determination of the concentration of fat and total acetone bodies. The hormone administration produced a marked lipemia but did not influence the rate of acetone body production. R.P.R.

316. Effectiveness of Blood and Hemin for Augmentation of Pituitary Gonadotropic Extracts in the Male. R. K. MEYER, W. H. McSHAN, AND L. E. CASIDA, Univ. of Wis., Madison, Wis. Soc. Expt. Biol. and Med. Proc., *52*, No. 2: 78. Feb., 1943.

Augmentation of the effect of pituitary gonadotropic extracts by the addition of hemin was demonstrated in immature male rats. The testes, seminal vesicles, and prostates were significantly heavier in rats receiving the pituitary extract in conjunction with hemin than in rats receiving an

equal amount of pituitary extract alone. No augmentation was observed in immature male pigeons or immature male chicks when hemin was added to the pituitary extract in the case of chickens or whole blood in the case of pigeons. R.P.R.

317. Hormonal Requirements for Pregnancy and Mammary Development in Hypophysectomized Rats. WM. R. LYONS, MIRIAM E. SIMPSON, AND HEBRERT M. EVANS, Univ. of Calif., Berkeley, Calif. Soc. Expt. Biol. and Med. Proc., *52*, No. 2: 134. Feb., 1943.

Sixty international units of purified lactogenic hormone and 10 international units of estrone injected daily ensured successful implantation in rats hypophysectomized and injected from the day of sperm. These two pure substances, however, in the doses used did not adequately substitute for the intact pituitary of a pregnant rat since only about one-half of the estimated number of implantations were found to have developed normally through mid-pregnancy. Seventeen out of 29 rats showed lobule-alveolar growth of the mammary glands equaling that of normal mid-pregnancy. Twenty of the 29 rats lost in body weight. R.P.R.

318. Influence on Growth of Thyroactive Iodocasein. MARVIN KOGER, E. P. REINEKE, AND C. W. TURNER, Univ. of Missouri, Columbia, Mo. Soc. Expt. Biol. and Med. Proc., *52*, No. 3: 236. March, 1943.

Virgin mice weighing from 13 to 15 gm. at the beginning of the experiment received thyroactive iodocasein by either oral or subcutaneous administration. Their gains in body weight and in length were compared with those of non-treated mice. The treated mice gained more in body weight and length than their corresponding controls. The experimental animals gained from 16 to 23% more in weight and an average of 28% more in length than the untreated controls. R.P.R.

319. Influence of Pregneninolone and Pregnenolone on Spermatogenesis in Hypophysectomized Adult Rats. J. H. LEATHEM AND B. J. BRENT, Rutgers Univ. and Roche-Organon, Inc. Soc. Expt. Biol. and Med. Proc., *52*, No. 4: 341. April, 1943.

Pregnenolone was found to be capable of maintaining spermatogenesis for at least 20 days after hypophysectomy. This steroid, however, did not influence the seminal vesicles since the decrease in seminal vesicle weight was similar to that observed in the hypophysectomized controls. Ten mg. of pregnenolone given in a single injection did not influence uterine weight or the adrenal X-zone of the spayed mouse. Pregneninolone exhibited androgenic activity but failed to maintain spermatogenesis in the hypophysectomized rat. R.P.R.

320. Lactogenic Hormone Content of the AP of the Pigeon. VICTOR HURST, JOSEPH MEITES, AND C. W. TURNER, Univ. of Missouri, Columbia, Mo. Soc. Expt. Biol. and Med. Proc., *53*, No. 2: 89. June, 1943.

The lactogen content of the anterior pituitary of two types of pigeons, the common and the White King, of both sexes were compared. Pituitaries of male pigeons contained $\frac{1}{2}$ to $\frac{1}{3}$ as much lactogen as the female. The pituitary of the common female pigeon contained as much or more lactogen than did that of the White King. Per 100 gm. of body weight the pituitary of the White King contained only $\frac{1}{2}$ to $\frac{2}{3}$ as much lactogen as the pituitary of the common pigeon. R.P.R.

321. Response of Hypophysectomized Immature Male Rats to Pregnant Mare Serum. J. H. LEATHEM, Rutgers Univ., New Brunswick, N. J. Soc. Expt. Biol. and Med. Proc., *53*, No. 2: 209. June, 1943.

Pregnant mare serum maintained testis weight and increased the seminal vesicle weight in hypophysectomized rats treated for a 5-day period. R.P.R.

322. Limited Effects of Certain Steroid Hormones on Mammary Glands of Hypophysectomized Rats. J. F. SMITHCORS AND S. L. LEONARD, Cornell Univ., Ithaca, N. Y. Soc. Expt. Biol. and Med. Proc., *54*, No. 1: 109. Oct., 1943.

Male and female rats of the Long-Evans Strain, from 26 to 38 days of age, and weighing from 50 to 98 gm. at the time of hypophysectomy were used. Rats were either injected immediately or 4 to 8 days after operation with progesterone alone or in combination with estrogen and with desoxycorticosterone acetate. The mammary glands were stimulated with progesterone and estrogen, either alone or in combination, only when treatment was begun immediately. The effects of the combined hormones indicated a summation of the individual responses. Desoxycorticosterone did not induce mammary gland growth. R.P.R.

323. Local Maintenance of Spermatogenesis in Hypophysectomized Rats with Low Dosages of Testosterone from Intratesticular Pellets. SAMUEL DVOSKIN, Columbia Univ. Soc. Expt. Biol. and Med. Proc., *54*, No. 1: 111. Oct., 1943.

Testosterone pellets were placed just beneath the tunica albuginea of the testis of rats of the Long-Evans strain at the time of hypophysectomy. The absorption of testosterone was slowed by mixing with cholesterol in varying proportions. Minute dosages of testosterone caused a regional maintenance of spermatogenesis. Such dosages were much less than those necessary for

complete maintenance of the sexual accessory glands. The direct action of testosterone on the testis in the maintenance of spermatogenesis was demonstrated. R.P.R.

324. Prolongation of Pseudopregnancy by Induction of Deciduomata in the Rat. B. H. ERSHOFF AND H. J. DEUEL, JR., Univ. South. Calif., Los Angeles, Calif. Soc. Expt. Biol. and Med. Proc., *54*, No. 2: 167. Nov., 1943.

Fifty-six female rats which were running normal estrous cycles and which were 3 to 4 months of age were used for experimental purposes. The induction of pseudopregnancy followed by the induction of deciduomata resulted in prolongation of pseudopregnancy to the 22nd day. This complete pseudopregnancy occurred in the absence of fetal tissue and was associated with the presence of metrial glands, maintenance of corpora lutea, and inhibition of follicular development. Mammary gland development was similar to that seen during incomplete pseudopregnancy and after the 12th day regression occurred. R.P.R.

325. Electron Microscope Study of Sperm. M. R. B. BAYLOR, A. NALBANDOV, AND G. L. CLARK. Noyes Chem. Lab. and Univ. of Ill., Urbana, Ill. Soc. Expt. Biol. and Med. Proc., *54*, No. 2: 229. Nov., 1943.

Semen samples were obtained from fertile bulls which were routinely used for artificial insemination. Fresh, unstained, and unfixed samples of spermatozoa were then studied under the electron microscope. These studies showed that the anterior portion of the sperm head was always enveloped by a protoplasmic cap which appeared damaged or disappeared if sperm were stained or fixed. The tail was found to end in a brush which consisted of many free and very long filaments. It also seemed likely that the axial filament consisted of a bundle of fine fibers rather than a single relatively thick thread. R.P.R.

326. Alterations in Mammary Structure Following Adrenalectomy in the Immature Male Rat. CHARLES F. REEDER AND S. L. LEONARD, Cornell Univ., Ithaca, N. Y. Soc. Expt. Biol. and Med. Proc., *55*, No. 1: 61. Jan., 1943.

Removal of the adrenals in either normal or castrated immature male rats resulted in an increase in the number of lateral buds of the mammary glands. In some cases increased end bud growth was noted, particularly when body growth was not markedly inhibited. Estrogen injection into adrenalectomized rats caused either dilatation of these stimulated lateral buds or further increased their number above that of the injected unoperated controls. R.P.R.

MISCELLANEOUS

327. Insect and Rodent Control in Dairy Plants. DWIGHT M. DELONG, Dept. of Zool. and Ent., Ohio State Univ., Columbus, Ohio. Milk Plant Monthly, *33*, No. 3: 60–61. March, 1944.

The author briefly describes effective methods of curtailing plants infested with roaches, flies, mites, dermestids, ants and rodents. G.M.T.

328. Giving Musca the Air. JERRY ZICH. Milk Plant Monthly, *33*, No. 3: 60–61. March, 1944.

The common house fly, *Musca domestica*, not only is a nuisance but a menace to public health, and, therefore, should be excluded from all dairies. By installing a blower over doors and above other openings to dairy buildings, a strong current of air may thus be provided which serves as a barrier for flies, which otherwise would enter the building. Small diagrams are given showing the effective arrangements of such installations. G.M.T.

329. The Most Important Factor in the Economic and Social Structure of the Nation. CHRIS L. CHRISTENSEN. Milk Plant Monthly, *33*, No. 1: 21–25. Jan., 1944.

Milk production has undergone a steady expansion for more than a decade. This expansion is wholly justified in the present light of nutritional value of milk and its products. The American consumer has never had sufficient milk or other dairy foods needed for good health. Dairying's future lies in translating this human need into an economic demand and a nutritional appreciation for the vast quantity of milk and milk products so that all efficient dairy operations may receive fair and equitable returns. Both the producer and manufacturer have certain obligations in further development of dairying. The dairyman needs to pay more attention to the economics of production in making use of abundant and high quality feed by use of alfalfa, improved strains and new varieties of grains, renovation of blue grass pastures, establishment of alfalfa-brome grass pastures, and employment of commercial nitrogen fertilizers. In addition, he must consider improved herd management and dairy breeding as well as improved dairy barns. The dairy manufacturer must effect economy in the operation of cheese factories, evaporating plants, creameries and other processing plants, as well as savings in handling and distributing of fluid milk and other dairy products. He must try to utilize total milk production, the solids of skim milk, as well as the fat. The dairy industry in general must promote research to expand our present knowledge of dairy products and must expand this basic educational program. The dairy industry, a major branch of agriculture, and therefore a major American industry, can prosper only when other industries prosper. G.M.T.

330. The Place of the Dairy Industry in Post-War America. CHRIS L. CHRISTENSEN, Vice-Pres., Celotex Corp. Certified Milk, *19*, No. 215: 7. March, 1944.

See Abstract 138, JOUR. DAIRY SCI., **27**, No. 4: A66. 1944.

H.G.L.

331. Forward Thinking in the Dairy Industry. W. A. WENTWORTH, The Borden Co., New York, N. Y. Milk Dealer, *33*, No. 7: 96–106. April, 1944.

There are two phases to consider in planning for the dairy industry—war and post-war. The problem of supplying the greatest demand for all dairy products ever experienced is the biggest war problem and will probably be the biggest early post-war problem. Charts and tables showing changes in consumption and utilization of milk for various products are included and discussed in this article. Factors to consider in both war and post-war planning in the dairy industry are: (1) the influence of national income upon the per capita consumption of different dairy products with their relative shifts dependent upon maintenance of income; (2) rehabilitation needs of Europe and the world will influence demands for dairy products and particularly fluid milk; (3) cooperation rather than competition whereby dairy products will supplement and not supplant each other should be a factor to consider; (4) a realization of proper price relationship of all dairy products to each other and to other commodities is essential to post-war growth and maintenance of all phases of dairy manufacturing; (5) the effect of Government price fixing, both ceiling and floor prices, needs to be considered in war and post-war thinking; (6) modification and unification of public health regulations whereby needless inspections can be eliminated, together with reciprocal acceptance of high-grade standards should benefit both consumers and the industry; and (7) the retention of wartime economies of procurement, processing and delivery of milk and all dairy products is vital to continued success of the dairy industry. C.S.T.

332. Looking Ahead with the Dairy Industry. O. E. REED, Chief of Bur. of Dairy Indus., U.S.D.A. Milk Dealer, *38*, No. 8: 48–50. May, 1944.

With the advent of war two things stand out: (1) the demonstrated ability of the industry to expand, and (2) the increased civilian demand for dairy products. Despite this increased demand, nutritionally as a nation we need to consume 275 quarts of milk or its equivalent per capita per year. The present average, however, is estimated at 231 quarts a year, indicating a 19% increase in consumption of dairy products to satisfy nutritional needs. Good quality of all dairy products and efficiency in their production

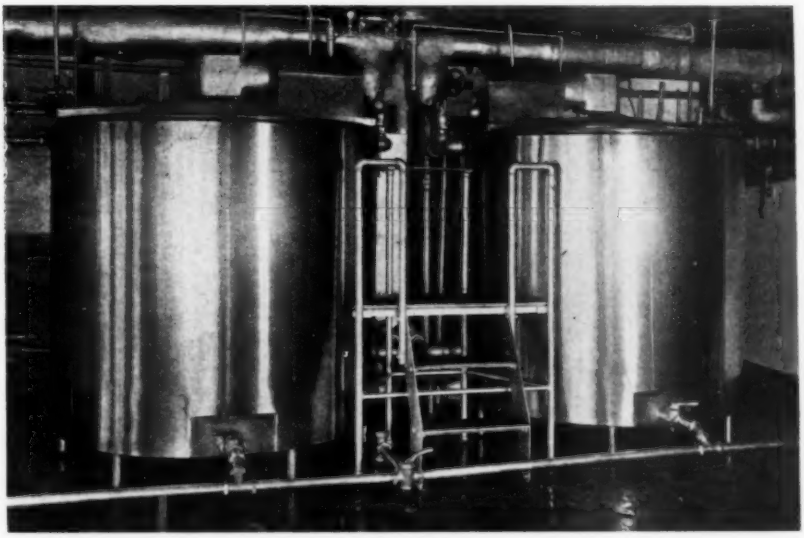
and distribution will be essential to supply increased consumptive demands, and both factors must be continually stressed as post-war problems. Fluid milk as such, particularly in the heavily populated northeastern section of the United States, will retain its present market preference provided its price is kept in line with competitive products. C.S.T.

333. Postwar Outlook for the Dairy Industry. DONALD E. HIRSCH, Farm Credit Admin. Natl. Butter and Cheese Jour., *35*, No. 6 : 26. June, 1944.

The federal government will probably establish price "floors" on dairy products to prevent sudden price declines. Such declines, which would be the logical effect of surpluses, may be avoided in part by education of consumers to the merits of dairy products in their numerous forms. The success or failure of dairy organizations will hinge on their ability to evaluate properly the significance of certain industrial changes and developments such as in the efficiency of utilizing milk solids, operating economies, plant flexibility, direct marketing to consumers, standards of quality for raw material and manufactured products, methods of establishing prices, artificial trade barriers and farming methods. Many organizations in industry can meet postwar problems better by reorganization, particularly cooperatives, by improving relations with patrons and customers and by sponsoring research and using the results to educate consumers. The outlook for the dairy industry is, on the whole, not unfavorable if all of its members keep informed and ready to make necessary adjustments. W.V.P.

WORLD'S No. 1 VAT

. . . for finest butter flavor and quality



Creameries using the Mojonnier Stainless Steel Cream Vat acclaim it the most outstanding unit on the market. Here are a few reasons why:

1. Its ability to maintain perfect temperature control in *heating, holding* and *cooling*—a most important factor in the obtaining of a fine butter flavor.

2. All operations can be performed in *one* unit—receiving the cream, following through with the exacting steps necessary to the production of a quality butter, to storing the cream for the churn. Thus, only *one* stickage loss.

3. Cleaning is surprisingly simple as there are no stuffing boxes and the interior is free from coils—just a one-piece, all-welded and polished mirror-like stainless steel cup that cleans quickly and easily.

4. Its single shell (not insulated) cover, which eliminates all of the hazards of a heavy cover while at same time providing even temperature holding.

5. And *efficiency*—users say without exception, "it's the quickest by far for heating and cooling."

All told, the many exclusive advantages of this Stainless Steel Vat make it well worth investigating for your operation. Write us today for complete details.

MOJONNIER BROS. CO. 4601 W. OHIO STREET CHICAGO 44, ILLINOIS

Mojonnier CREAM VAT

Your advertisement is being read in every State and in 25 Foreign Countries

AMERICAN DAIRY SCIENCE ASSOCIATION

INCORPORATED IN THE DISTRICT OF COLUMBIA

Officers

President ..... A. C. RAGSDALE, Columbia, Missouri
Vice-President ..... J. A. NELSON, Bozeman, Montana
Secretary-Treasurer ..... R. B. STOLTZ, Columbus, Ohio
Editor ..... T. S. SUTTON, Columbus, Ohio
Director ..... P. A. DOWNS, Lincoln, Nebraska
Director ..... J. A. LINN, Manhattan, Kansas
Director ..... P. H. TRACY, Urbana, Illinois
Director ..... C. L. BLACKMAN, Columbus, Ohio
Director ..... R. B. BECKER, Gainesville, Florida
Director ..... W. E. PETERSEN, St. Paul, Minnesota
Director ..... A. C. DAHLBERG, Ithaca, New York

Officers of Sections

DAIRY PRODUCTION SECTION

Chairman ..... GLEN SALISBURY, Ithaca, N. Y.
Vice-Chairman ..... DWIGHT SEATH, University, La.
Secretary ..... GEORGE WISE, Clemson, S. C.

DAIRY MANUFACTURING SECTION

Chairman ..... L. K. CROWE, Lincoln, Nebraska
Vice-Chairman ..... C. L. HANKINSON, Milwaukee, Wis.
Secretary ..... N. S. GOLDING, Pullman, Washington

DAIRY EXTENSION SECTION

Chairman ..... FLOYD ARNOLD, College Park, Maryland
Vice-Chairman ..... W. T. CRANDALL, Ithaca, New York
Secretary ..... E. H. LOVELAND, Burlington, Vt.

Officers of Divisions

SOUTHERN

Chairman ..... R. B. BECKER, Gainesville, Fla.
Vice-Chairman ..... J. P. LAMASTER, Clemson, S. C.
Secretary-Treasurer ..... F. H. HERZER, State College, Miss.

EASTERN

Chairman ..... H. C. MOORE, Durham, N. H.
Vice-Chairman ..... A. I. MANN, Storrs, Conn.
Secretary-Treasurer ..... L. R. DOWD, Storrs, Conn.

WESTERN

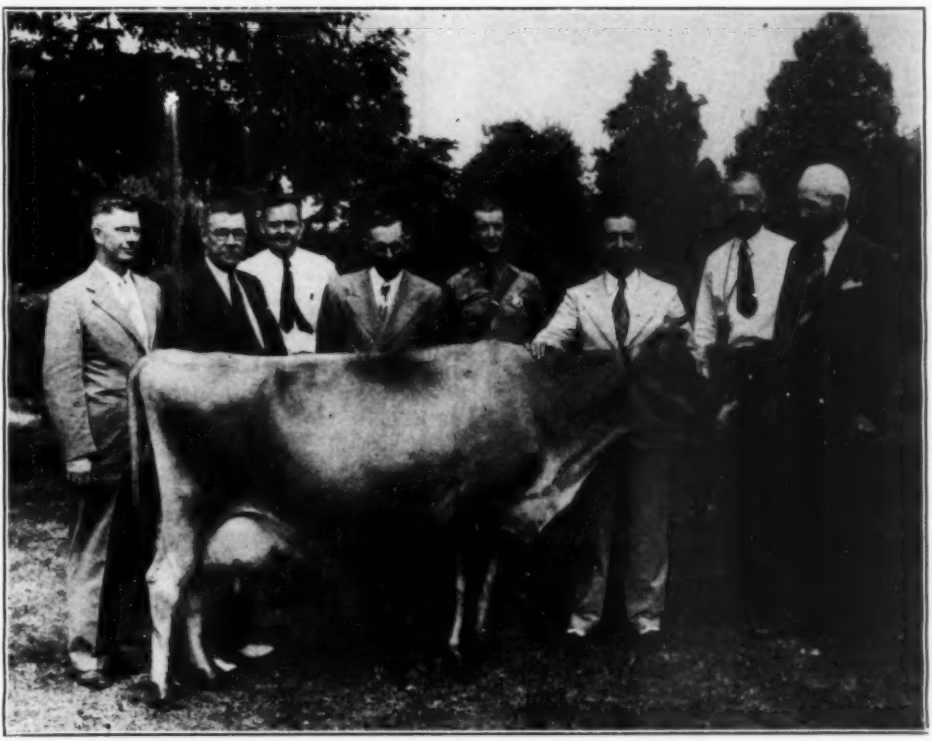
Chairman ..... N. S. GOLDING, Pullman, Wash.
Vice-Chairman ..... S. W. MEAD, Davis, Calif.
Secretary-Treasurer ..... H. P. EWALT, Corvallis, Oregon

The American Dairy Science Association was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

Membership. Any person is eligible to membership who is formally announced by an Agricultural College or Experiment Station, or by the Bureau of Dairy Industry of the United States Department of Agriculture or by the Canadian Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or any person filling a position of responsibility connected with the dairy industry who has had a college or university training in technical science, or any person filling a responsible position in the dairy industry of a professional character requiring a technical knowledge of dairying of a high order. The membership fee is $5.00.

The dues are $5.00 a year, $3.00 of which is for a year's subscription to the *Journal of Dairy Science*. Correspondence regarding membership and dues should be addressed to R. B. Stoltz, Ohio State University, Columbus, Ohio.

Great Jersey Cow Goes to College



Left to Right: Dr. N. D. Peacock, Vice-Director, Resident Instruction, College of Agriculture, University of Tennessee; Dr. C. E. Brehm, Dean, College of Agriculture, University of Tennessee; Professor S. A. Hinton, Assistant Professor of Dairying, University of Tennessee; Mr. J. J. Broome, Speaker, Tennessee House of Representatives; Sgt. J. C. Harris, Tennessee State Highway Patrol; Governor Prentice Cooper; Professor C. E. Wylie, Head, Department of Dairying, University of Tennessee; Dr. J. D. Hoskins, President, University of Tennessee.

GOVERNOR PRENTICE COOPER recently paid $3,500 to Crieve Hall Farm for Faithful Design Peeress, a Jersey Excellent. She was presented to the University of Tennessee, where her great inheritance and inspirational value to herd improvement will be given its greatest possible opportunity.

"Faithful" is a member of the family of more than 10,000,000 Jerseys owned in the United States.

The American Jersey Cattle Club
324 West 23rd Street
NEW YORK 11, NEW YORK
Your advertisement is being read in every State and in 25 Foreign Countries

INTO THE STRETCH!

WATCH the winner pull into the lead, and you see a great example of coordination and teamwork between trainer, jockey and thoroughbred.

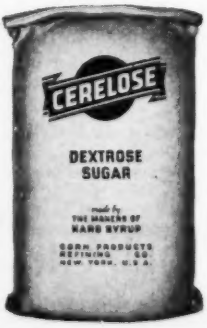
Coordinated teamwork between producer, processor and distributor is making dextrose sugar a winner, too.

As millions of consumers read current dextrose advertising, which is consistent, interesting and convincing, they respond by increasing their approval of products *rich in dextrose*—knowing that "dextrose on the label means food energy in the product."

CORN PRODUCTS SALES COMPANY
17 Battery Place, New York 4, N. Y.

Tune in
STAGE DOOR CANTEEN

Every Friday
10:30 to 11:00
P.M., E.W.T.
Columbia Network,
Coast-to-Coast



CERELOSE *is* DEXTROSE

FLAV-O-LAC FLAKES

THE CULTURE

of definitely better flavor & aroma-producing qualities.

The standard with foremost operators, agricultural schools & colleges.

FLAV-O-LAC FLAKES (shown) produce a quart of the finest starter on a single propagation. Single bottles $2.00.

SPECIAL FLAV-O-LAC FLAKES "40" produce 40 quarts of starter on a single propagation. Single bottles $5.00

Free Culture Manual of Fermented Milk Products on request.

Pioneers in Spectro-chemical, Chemical and Fluoro-photometric Determinations of Vitamins A, B_1, B_2, Nicotinic Acid, Pantothenic Acid, B_6, C & E in Dairy and Food Products. (Vitamin D excluded) inquiries invited.

THE DAIRY LABORATORIES
A DIVISION OF DALARE ASSOCIATES
23rd & Locust Sts., Phila., Pa.
BRANCHES
New York Baltimore Washington
See our catalog in Dairy Industries Catalog.

Better Bottle Washing With Oakite Bottle-Soak

Clean, cloudless, crystal-bright bottles that test LOW count . . . those are the "proved-by-performance" advantages that result when soaker, hydro-soaker or pressure type bottle-washing machines are charged with

OAKITE BOTTLE-SOAK
(Protected by U. S. Patent)

Providing effective detergent action and free-rinsing properties, this time-tested material, in addition, has an EXCLUSIVE bacteria-destroying ingredient that helps keep washing solutions sanitary by inhibiting bacterial growth and retarding rancidity. The use of Oakite Bottle-Soak also eliminates troublesome foaming . . . retards lime-scale formation on conveyors. FREE 20-page booklet contains interesting details. Write for YOUR copy TODAY!

OAKITE PRODUCTS, INC.,
Dairy Research Division,
16G THAMES STREET, NEW YORK 6, N. Y.
Technical Service Representatives Located in All Principal Cities of the United States and Canada

OAKITE Trade Mark Reg. U.S. Pat. Off. **CLEANING**

THE HOLSTEIN-FRIESIAN Association of America was established in 1885 for the purpose of improving the Holstein-Friesian breed of cattle, preserving and publishing the pedigrees and production records and promoting the best interests of the breed in general.

But Our Interests Have Always Had a Much Wider Scope

We have always been interested in contributing as much as possible to the general progress and improvement of the Dairy Industry as a whole through our program of breed improvement.

That is why our members recognized from the beginning (1885) the value of testing cows for production and why we adopted the Babcock test (in 1894) as a means of determining the amount of butterfat in milk. More than 69,000 production records have been recorded by Advanced Registry and more than 118,000 records have been completed in our Herd Test program to date.

That is why we recognized the need for and established (in 1923) the True Type or "ideal" Holstein cow and bull. That is why we established our Herd Inspection and Classification Program (in 1929) in which more than 27,000 animals have been given an official rating.

That is why we believe in the utmost cooperation with other purebred dairy cattle associations, with research and extension workers in colleges and experiment stations, and with dairymen's groups which are designed to assist in the progress of the Dairy Industry as a whole.

THE HOLSTEIN–FRIESIAN ASSOCIATION OF AMERICA
BRATTLEBORO, VERMONT

Established in 1885 **33,000 Members**

Our bulletin 30c is fully descriptive—Write for it.

"THE GAULIN TWO STAGE HOMOGENIZER AND HIGH PRESSURE PUMPS have met the Dairy Industries needs for a durable, heavy duty and economical machine, since 1909. Homogenizers and Pumps are still available with proper priority ratings, as well as repair and replacement parts. Both machines are strictly sanitary and are approved by all leading health boards. The Homogenizers are built in sizes from 75 gallons to 2000 gallons per hour, and the High Pressure Pumps are custom built to suit individual needs. The Gaulin is the ideal, all-purpose machine for fluid milk, ice cream, evaporated milk, condensed milk and spray-drying milk or eggs."

MANTON-GAULIN
TWO STAGE
HOMOGENIZER

THE MANTON-GAULIN MFG. CO., INC.
7 CHARLTON STREET **EVERETT, MASS., U.S.A.**

Your advertisement is being read in every State and in 25 Foreign Countries

The LAST *of a series discussing how Nature and Man together develop our finer foods.*

GROUNDWORK FOR THE *Two Billion Gallon Ice Cream Year*

Before ice cream volume reaches that high altitude figure a lot of good, sound groundwork will have to be done.

We, here at David Michael's, have had a most interesting opportunity to hear what many authorities are thinking about on this subject.

As a result of the series of advertisements we have been publishing in this magazine, ice cream leaders and Federal and State food authorities have volunteered some advice for the industry. They have shown considerable interest in these detailed discussions of taste and flavor and ice cream. And now they say the industry's No. 1 job is TO TELL THE PUBLIC more about ice cream and all of its ingredients.

It isn't just a matter of saying ice cream tastes good. The time has come when the public has to be given a new interest in this wonderful food. And the way to kindle that new interest is to tell the fascinating facts that make modern, American ice cream what it is.

Few food products have so many grand stories to tell. In every case the details reveal how Science and Nature combine to produce a finer and finer result.

There is often a tendency to think the public isn't interested. But the facts all about us contradict that thought. The lively consumer interest in electronics and television and synthetics is proof positive that the thirst for knowledge on the part of the consuming public is real and genuine.

Members of our armed forces are consuming twice as much ice cream as the civilian stay-at-homes. The two billion gallon goal is not too much to expect for an alert industry in the post-war period. It can be done. But we must get out and tell the FULL story of ice cream, with all its progressive developments.

* * *

In recent articles, David Michael & Company has published some of the wonders of the ice cream business—brief stories about processing and vanilla flavorings and taste. We have been told that these have helped to stimulate the imagination of men within the industry. As a further aid in this program to pave the way for increased sales by education, we are preparing a small brochure which will contain some ice cream stories that should interest your consuming public. These will go forward to you as another demonstration of the constant cooperation between our company and yours.

Published in the interest of continued progressive thinking in the food industries, by

DAVID MICHAEL & CO.

makers of Mixrean and other powdered vanilla products

Front and Master Streets, Philadelphia 22, Pa.

Your advertisement is being read in every State and in 25 Foreign Countries

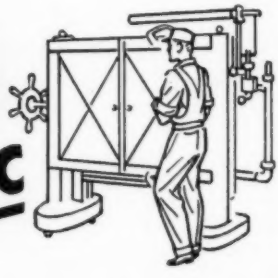
SANITATION TIPS
BY THE DIVERSEY D-MAN

HOW TO REMOVE
MILK
DEPOSITS

ON THE FARM WITH
DICOLOID

A dry, concentrated product, Dicoloid is readily applied in paste form with a wet brush. Penetrates, dissolves and suspends milkstone deposits through its powerful wetting and cleaning action . . . its casein and mineral dissolving properties. Safe to hands or utensils. Agriculture Experiment Stations have shown that milkstone is often the cause of high bacteria counts in milk.

IN THE PLANT WITH
DILAC



Diversey Dilac has been specially developed to quickly and effectively remove the stubborn contamination that forms daily on short-time, high temperature pasteurizing units. . . . Softens and dissolves milkstone so it can be completely removed with a minimum of time, labor and material. Write for technical bulletin "Milkstone Control," **THE DIVERSEY CORPORATION**, 53 W. Jackson Blvd., Chicago 4.

"HANSEN'S"
Dairy
Preparations

Cheese Rennet and Color
Annatto Butter Color
Certified Butter Color
Ice Cream Color
Lactic Ferment Culture
Bulgarian Culture
—★—
Cheese Bandages, Circles
Press Cloths
Odorless Dairy Fly Spray
Testing Solutions
Rennet Tests

CHR. HANSEN'S LABORATORY, INC.
MILWAUKEE, WISCONSIN

Marschall
RENNET and COLOR

For best results in the cheese vat, specify Marschall's. High in strength, pure and uniform.

MARSCHALL
DAIRY LABORATORY, INC.
MADISON 3, WISCONSIN

Your advertisement is being read in every State and in 25 Foreign Countries

We're fighting

BACTERIA

We are glad to say that R. G. Wright is again making some can and bottle washers and other dairy equipment.

Ever since Pearl Harbor most of our facilities have been devoted to direct war production. The lessons we have learned during that time and the new production techniques developed will mean much to the dairy industry in terms of still better R. G. Wright equipment.

Our technical experience is always at your service. Do you have any immediate problems? Let's talk them over.

R. G. Wright
Company, Inc.
BUFFALO 14, N. Y.

Can Washers . . Bottle Washers . . Pasteurizers . . Surface Coolers . . Weighing and Receiving Equipment . . Sanitary Pumps . . Conveyors and Other Milk Plant Equipment.

Copies of the 20-Year Index covering Volumes I to XX, inclusive are available at the following prices:

MEMBERS

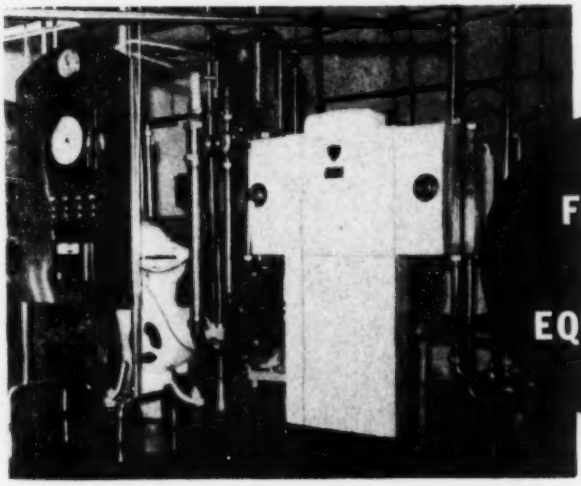
Cloth Bound **$2.35**
Paper Bound **2.00**

NON–MEMBERS

Cloth Bound **$5.50**
Paper Bound **5.00**

Your advertisement is being read in every State and in 25 Foreign Countries

BEST FOR MANY JOBS....



FULL-FLO PLATE EQUIPMENT

Illustrated above: CP Full-Flo Plate HTST Pasteurizing Unit used on market milk at Franklin Cooperative Creamery Co., Minneapolis.

Hundreds of the finest dairy products plants in America and Canada have found the CP Full-Flo Plate Unit the most versatile and efficient equipment that money can buy. The "check list" at right will give you some idea of the variety of products being processed with this equipment.

Whatever your heat exchange problem, whether heating, cooling, preheating, regeneration or High Temperature Short Time pasteurizing, you'll find that CP Full-Flo Plate Equipment is tops. Ask for illustrated Bulletin E-5 which thoroughly explains the many exclusive CP advantages.

- ☐ **Heating and cooling cream.**
- ☐ **HTST pasteurizing of whole milk.**
- ☐ **HTST pasteurizing of market cream.**
- ☐ **HTST pasteurizing of cheese milk.**
- ☐ **Cooling butter wash water.**
- ☐ **Heating and cooling skim and whole milk for spray drying.**
- ☐ **Cooling evaporated milk.**

ARMY E NAVY

Creamery Package Fort Atkinson Plant

THE CREAMERY PACKAGE MFG. CO.

1243 W. Washington Blvd., Chicago 7, Illinois

Branches: Atlanta — Boston — Buffalo — Chicago — Dallas — Denver — Kansas City — Los Angeles — Minneapolis — New York — Omaha — Philadelphia — Portland, Oregon — Salt Lake City — San Francisco — Seattle — Toledo — Waterloo, Iowa

CREAMERY PACKAGE MFG. CO. OF CANADA, LTD.
267 King St., West, Toronto 2, Ont., Canada

Your advertisement is being read in every State and in 25 Foreign Countries

DIFCO

Culture Media for Examination of MILK *and* DAIRY PRODUCTS

for Plate Counts

Bacto-Tryptone Glucose Extract Agar is recommended for routine plate counts of bacteria in milk. This medium conforms to all requirements of "Standard Methods for the Examination of Dairy Products" of the American Public Health Association, except that it does not contain skim milk.

Bacto-Proteose Tryptone Agar is recommended for determinations of the total bacterial plate count of certified milk. This medium is prepared according to the specifications of "Methods and Standards for Certified Milk" of the American Association of Medical Milk Commissions.

for Detection of Coliform Bacteria

Bacto-Violet Red Bile Agar is widely used for direct plate counts of coliform bacteria. Upon plates of this medium accurate counts of these organisms are readily obtained.

Bacto Brilliant Green Bile 2% and

Bacto-Formate Ricinoleate Broth are very useful liquid media for detection of coliform bacteria in milk. Use of these media is approved in "Standard Methods."

for Detection of Molds

Bacto-Potato Dextrose Agar is an excellent medium for detection and enumeration of molds and yeasts in butter and other dairy products. The formula of this medium corresponds exactly with that specified in "Standard Methods."

Bacto-Malt Agar is also widely used for determinations of the mold and yeast count of dairy products and for control of the sanitary conditions of manufacture.

for Cultivation of Lactobacilli

Bacto-Tomato Juice Agar and

Bacto-Trypsin Digest Agar support luxuriant and characteristic growth of *Lactobacillus acidophilus*, and are well adapted for use in establishing the number of viable organisms in acidophilus products. These media are also widely used for estimation of the degree of implantation by *L. acidophilus*.

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media.

DIFCO LABORATORIES
INCORPORATED
DETROIT, MICHIGAN

Your advertisement is being read in every State and in 25 Foreign Countries